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Follicular Lymphoma and Mantle Cell Lymphoma

Pathobiology, Diagnosis and Treatment

Humana Press

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Preface

The book is a sequel to the earlier written book on *Chronic Lymphocytic Leukemia*. This book is dedicated to Follicular Lymphoma and Mantle Cell Lymphoma. Hematologic malignancies have traversed a long journey in delineation from primarily morphologically defined entities to phenotypically qualified entities and now to molecularly defined identities that mimic normal B cell differentiation. The pathobiology lays foundations for definition, classification, and treatment, the latter based on identifying targetable mutations. The molecular mechanisms cover the spectrum of topics covered in immunology, bioinformatics, biochemistry, and molecular biology. The aim of this book is to bring the basic science adjacent to the applied science and highlight major works that contributed significantly to the understanding of these two lymphomas. There is significant focus on the biology of normal B cell development that forms the basis for understanding the pathobiology of these lesions. What goes wrong, where, why, and how? This book is designed to provide a concise and yet comprehensive summary of traditional and newer entities and the science that explains the same. The hematopathologists and dermatopathologists have written the pathobiology sections and hematologists address treatment. I hope this book is an approachable read for residents and fellows and clinically relevant to practicing pathologists and hematologists.

As always, these writes do not happen without the unwavering support of family. We are hugely indebted to them as they forgive our absence from meals and household chores as we work through these writes. Thank you.

Lebanon, NH, USA — Prabhjot Kaur, MD

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Chapter 1
Follicular Lymphoma: Definition, Epidemiology, Pathobiology – Lymphomagenesis, Morphology, Variants, Immunophenotype, Prognostic Factors, Transformation

Prabhjot Kaur

Introduction, with a Case

Case

A 60-year-old woman presented with lower abdominal pain, anorexia, fatigue, significant weight loss, and lower extremity weakness. CT scan revealed enlarged lymph nodes in retroperitoneal, mesenteric, pelvic, right inguinal, porta hepatic, cervical regions, and lytic lesions in her thoracic, lumbar, and sacral spine. She was HIV negative.

The cervical lymph node biopsy showed an effaced architecture by irregular closely packed back-to-back follicles lacking polarization and with attenuated mantle zones. They were composed of small cleaved centrocytes, with rare centroblast, and did not show tingible body macrophages. The centroblast were not more than 3–4/HPF averaged over 10 high-power fields. The neoplastic lymphocytes were positive for CD20, CD10, Bcl6, Bcl2, and Ki-67 which showed a low proliferative fraction. No large cell transformation was seen. Based on these features, a diagnosis of follicular lymphoma grades I–II was made. The bone marrow biopsy was negative for lymphoma (Fig. 1.1).

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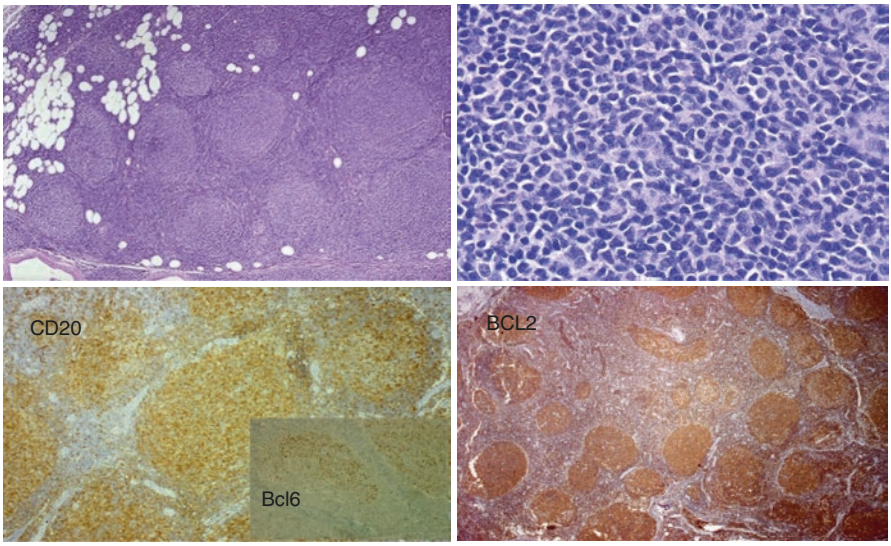

Fig. 1.1 Top left: lymph node effaced by a nodular back-to-back proliferation. Top right: neoplastic infiltrate comprises mostly of small cleaved lymphocytes

Definition

Follicular lymphoma (FL) is the most common type of indolent adult non-Hodgkin lymphoma (NHL) [1]. It is composed of cells derived from germinal center B cells that demonstrate IgV genes with extensive and ongoing somatic hypermutation (SHM) – the hallmark of germinal center cells [2, 3]. The lymphoma shows, at least partially, a follicular growth pattern and contains cells that are typically centrocytes and centroblasts. Lymphomas containing centrocytes and centroblasts with an entirely diffuse pattern may be included in this category. Lymphomas containing only centroblasts are considered evidence of progression to diffuse large B-cell lymphoma (DLBCL). Four variants are recognized: (1) in situ follicular neoplasia;(2) duodenal-type FL; (3) testicular FL; and (4) diffuse variant of FL. Approximately 90% of FL carry the chromosomal translocation t(14;18)(q32;q21) as a primary oncogenic event. This translocation juxtaposes the *BCL2* oncogene from 18q21 next to the immunoglobulin heavy chain (*IGH*) locus in 14q32. Primary cutaneous follicle center lymphoma and pediatric-type FL (PTFL) are biologically and molecularly distinct entities and therefore considered as distinct entities by the updated World Health Organization classification [1].

Epidemiology

Follicular lymphoma is a disease of adults and rarely seen in patients aged <18 years. The median age at presentation is the sixth decade of life with male to female ratio of 1:1.7. According to the Surveillance, Epidemiology, and End Results (SEER) Program, the number of new cases of follicular lymphoma, in the USA, was 2.7 per 100,000 men and women per year. [https://seer.cancer.gov/statfacts/html/follicular.html]. The number of deaths was 0.5 per 100,000 men and women per year with a median survival of 10 years. These rates are age-adjusted and based on 2012–2016 cases and deaths. The disease is most seen in Whites, followed by Hispanics, Blacks, Asians/Pacific Islanders, and Native Americans [4]. A higher incidence has been observed among individuals of Asian descent born in the USA than in those born in Asia, which supports an environmental component in the risk of FL in addition to genetic and ethnic factors [5]. Pediatric-type FL comprises <6.5% of childhood lymphomas, with a median age range of 7.5–11.7 years at diagnosis and a male to female ratio of 4:1 [6].

Genetic Susceptibility

FL has an increased incidence in patients with a family history of lymphoma in first-degree relatives [7]. Genome-wide association studies found that multiple single-nucleotide polymorphisms (SNPs) in human leukocyte antigen (HLA) class I and II genes on chromosome 6p21.3 significantly influenced FL risk. These findings highlight a central role for antigen presentation in FL pathogenesis. In addition, FL risk was strongly associated with homozygosity at HLA class II loci. The premise is that homozygosity at *HLA* loci reduces the diversity of peptides that can be presented that may include infectious agents, self-antigens for atopic or autoimmune conditions, and neoplastic cells. At present, there is a growing body of literature supporting that *HLA* heterozygotes are more resistant to infectious diseases, and accordingly *HLA* homozygotes are more susceptible to antigens (infection, immune, or neoplastic cells) [8, 9]. SNPs are localized near potential and proven oncogenes: *BCL2* at *18q21.3*, *CXCR5* at *11q23.3*, *ETS1* at *11q24.5*, and *PVT1* at *8q24*. These results suggest that germline variation in pathways involved in B-cell apoptosis, GC function, or modulations in FL cell–tumor microenvironment (TME) interactions influence disease progression [10].

Environmental Exposure Agricultural exposure to pesticides and herbicides has been associated with increased risk. In a case-control study, it was shown that farmers exposed to pesticides carried an extra burden of activated $t(14;18)^{+}$ B cells.

These cells recapitulate the hallmark features of developmentally blocked FL cells, with some displaying aberrant activation-induced cytidine deaminase activity linked to malignant progression. In a follow-up period of 9 years, the frequency of $t(14;18)^+$ B cells was much higher as compared to the control group, adjusted for age [11, 12].

Pathobiology

It is recommended that we review normal B-cell development before we delve into the pathobiology of follicular lymphoma (the next section), because follicular lymphoma cells pretty much manipulate this system to its advantage.

Normal B-Cell Development

The B-cell receptor (BCR), the signature that defines a B cell and its molecular pathway, are important to understand normal B-cell physiology and the aberrancies that result in neoplasia. Therefore we will start by briefly reviewing some basic immunology of the BCR and the B-cell receptor (BCR) signaling pathways that are critical to the development and maturation of normal B cells. This leads to biological responses including activation, tolerance, and/or differentiation, depending on the nature of the stimulus and the differentiative state of the B cell [13–15].

The B-cell receptor (BCR) is a transmembrane receptor protein located on the B-cell membrane with more than 10^4–10^5 copies per cell [16]. The BCR includes sIg (IgM, IgD) and the Ig-α/Ig-β heterodimer (CD79a and CD79b). The sIg (IgM, IgD), the basic unit of the antibody, is composed of two identical heavy chains and two identical light chains with variable amino terminal region and a constant carboxy-terminal region. The variable amino terminal is made of a unique and randomly determined antigen-binding site that is mediated via somatic gene recombination that occurs in the developing B lymphocyte and results in a highly diverse repertoire of immunoglobulins [17]. The IgH gene loci are located in chromosome 14q32.3 and contain many different variable (V), diversity (D), and joining (J) gene segments. There are about 80 VH segments (that include about 50 functional segments that can be grouped according to their homology in 6–7 VH subgroups/families), about 27 DH segments, and 6 functional J segments that undergo V–D–J rearrangement that generally starts with a D to J rearrangement followed by a V to D–J rearrangement. The most frequently used VH gene segments in normal and malignant B cells belong to the VH3 family (30–50%), VH4 family (20–30%), and VH1 family (10–20%), together covering 75–95% of VH usage [18]. Likewise the IgK light chain, locus on chromosome 2p11.2, and IgL light chain on chromosome 22q11.2 have many distinct V and J segments, without the D gene segment, that undergo V to J rearrangements. The rearrangements generally follow a hierarchical order; first the *IGH* genes rearrange, then *IGK*, potentially resulting in IgH/kappa

expression or should the kappa rearrangements be unproductive, and it is followed by *IGL* rearrangement leading to IgH/*lambda* expression. These require the presence of recombination-activating genes RAG1 and RAG2 that are involved in recognizing and cutting the DNA at the recombination signal sequences (RSS) located adjacent to the V, D, and J gene segments. The sequences between rearranging gene segments are generally deleted in the form of a circular excision product called B-cell receptor excision circle. The unique cell surface receptors are supported by the "one-cell-one-antibody" hypothesis that is based on allelic exclusion. This model proposes that a productive VHDJH rearrangement prevents an additional VH-to-DJH rearrangement [19]. This combinatorial repertoire is estimated to be ~2×10^6 Ig molecules. At this stage, the monomorphic pseudo-L chain (VpreB +λ5) is also synthesized, permitting the surface expression of the VHDJH –μ + VpreB +λ5 complex (pre-B-cell receptor). In addition, the enzyme terminal deoxytransferase may introduce additional nucleotides at the D–JH and VH –DJH junctions, thereby increasing diversity of the V regions of developing B cells that adds to the total repertoire estimated >10^{12}. These productive rearrangements on both heavy (IgH) and light (IgL) chains result in the formation of a BCR of IgM and/or IgD isotype. At this point the B cell can express individual IgM or IgD or both IgM and IgD. These B cells are considered naïve B cells and are ready to move out of the bone marrow to reach the lymphoid regions of the lymph node, spleen, or intestinal payers patches.

The mature B cells in peripheral lymphoid tissues can follow *two maturation pathways.*

Germinal Center Pathway The B cells can traverse the germinal center [20] where the follicular B cells respond to the antigens via T-cell help that induces V-gene mutations in germinal center B cells. The B cells that give rise to germinal centers initially have to be activated outside follicles with the help of interdigitating cells and T-cell help and start out as oligoclonal blasts. These blasts undergo massive clonal expansion and activate hypermutation in the immunoglobulin-variable (Ig-v)-region genes. The proliferating blasts, centroblasts, occupy the dark zone and give rise to centrocytes that fill the light zone. The light zone contains a rich network of follicular dendritic cells (FDC) that have the capacity to take up antigen and hold this on their surface for periods of more than a year. The antigen is held as an immune complex in a native unprocessed form; but the antigen may be taken up from FDC by B cells, which can process this and present it to T cells. Centrocytes appear to be selected by their ability to interact with antigen held on FDC. Those that cannot interact with the FDC undergo apoptosis. Centrocytes also get their survival signals by cross-linking centrocytes' surface Ig and signaling through their surface CD40. The centrocytes then acquire characteristics of memory and plasma cells [21].

Non-germinal Center Pathway The other pathway is via the marginal zone where marginal zone B cells respond to antigens, most often carbohydrates of encapsulated bacteria or viruses, without T-cell participation. T-cell-independent antigenic stimulation may or may not induce V-gene mutations in marginal zone B cells [22–

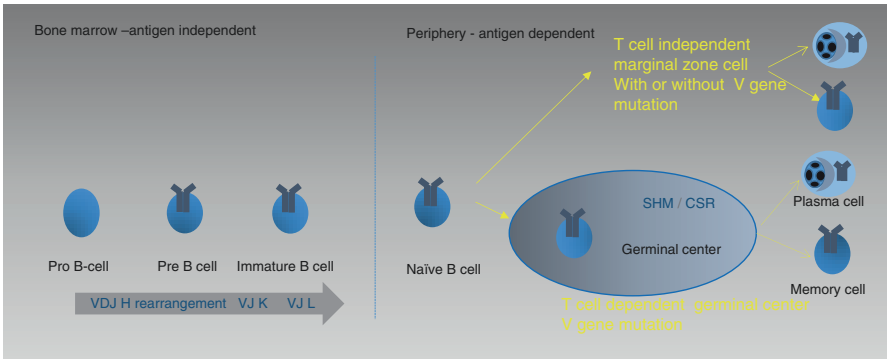

Fig. 1.2 Normal naive B cells undergo V(D)J recombination, and functional naïve B cells mature via two pathways in the periphery. In the germinal center, they bind to antigen via interaction with CD4+ T cells and follicular dendritic cells. They experience rapid proliferation and somatic hypermutation (SHM) and class-switch recombination (CSR). The second maturation pathway is via the marginal zone and is T-cell independent. They may or may not undergo V-gene mutation. Memory cells without V-gene mutations that derive from T-cell-independent stimulation are often referred to as antigen-experienced B cells, to distinguish them from memory cells with somatic mutations

26]. Both the T-cell-dependent and T-cell-independent pathways lead to either plasma cells or memory cells. Memory cells without V-gene mutations that derive from T-cell-independent stimulation are often referred to as antigen-experienced B cells, to distinguish them from memory cells with somatic mutations [27] (Fig. 1.2).

Antibody Diversity

Antibody diversity is produced during both stages of B-cell development, that is, in pre-B cells in the bone marrow, omentum or liver, and in the mature B cells in the germinal centers of lymph nodes. In *pre-B cells*, rearrangement of variable (V), diversity (D), and joining (J) gene segments result in combinatorial diversity, and junctional diversity occurs at the site of V-D-J gene combination (where deletion or nontemplated insertion of nucleotides occurs by nTDT). This produces the primary repertoire of immunoglobulin (Ig) receptors.

In mature B cells, Ig receptors undergo somatic hypermutation (SHM) both during affinity maturation (AM) and class-switch recombination (CSR), to produce the secondary repertoire of antibodies. In this SHM process, many non-random, single-base changes result in diversification of the *Ig V* repertoire [28, 29]. The process is called hypermutation as the rate of V-region mutations, $\sim 10^{-3}$ per base pair in one generation, is roughly a million times greater than normal somatic mutation. AM is a process by which T-follicular helper cell-activated B cells produce antibodies with increased affinity for antigen during the course of an immune response. With each exposure to an antigen, the centrocytes undergo more mutations in the IG receptors

in order to create receptors that will bind the antigen with stronger avidity. Centrocytes that fail to do this die. CSR is a mechanism by which B cells change their isotype from IgM/or IgD to IgG, IgA, or IgE. During this process, the constant portion of the antibody heavy chain is changed, but the variable region of the heavy chain stays the same. Each time the constant portion is attached to the variable portion, mutations are introduced at the junctions that increase their diversity [30].

Somatic hypermutations (SHM) are generated by *deamination.* These are introduced by the enzyme activation-induced (cytidine) deaminase (AID). AID produces diversity by converting cytosine to uracil which removes an amine group from cytosine to generate a uracil. The cytosine: guanine pair changes to a uracil: guanine pair. Since uracil residues are not normally found in DNA, these mutations are "corrected" and what follows is more mutations [31, 32]. There are series of enzymes and DNA polymerases that function in tandem to generate mutations; otherwise it is the nature of DNA to repair precisely. In brief, once cytosine is converted to uracil by AID, there are several pathways that can follow. One is replication – here high fidelity DNA-replicating enzymes (polymerases) recognize U as thymine (T) and pair it with adenine (A). This essentially results in a transition from G-to-A and C-to-T (remember A and G are purines and T and C are pyrimidines; normal pairing is A-T and G-C.). Another pathway is removal of uracil by uracil-DNA glycosylase (UNG), to create an abasic site. Low fidelity polymerases bypass the resulting gap during replication, filling it more or less at random (can result in both transition and transversion). In another "error-prone base-excision repair," the gap may be incorrectly filled in by DNA polymerase, which erroneously inserts thymine opposite guanine. In the "short-patch repair synthesis," polymerase can fill in the gap and synthesize several bases by strand displacement opposite adenines and thymines. These involve the mismatch-repair proteins MSH2 and MSH6 and polymerase [33, 34]. In summary, the resulting U:G mismatch can be alternatively recognized and processed by base excision repair (BER) or mismatch repair (MMR) pathways, leading either to point mutations, in the case of SHM, or to double-strand breaks (DSBs) followed by a recombination reaction, in the case of CSR [32, 35, 36].

Another less understood is the *strand-biased mutation signature* [37]. If mutations were random with equal frequencies on both strands, then transition mutations A → G on one of the strands should be of the same frequency as of its Watson-Crick reciprocal complement, T → C, on the *same* strand. However, there is data to suggest that mutations from A sites (A → T, A → C, A → G) were at least two- to threefold excess over mutations at T sites (T → A, T → C, T → G), an imbalance not explainable by the base composition in the mutated *V(D)J* target region analyzed [37]. Analysis of somatic hypermutations in xeroderma pigmentosum variant patients (who are deficient in the enzyme polymerase-eta) by Gearhart PJ et al. further shows that the striking A-T mutations occur more on the non-transcribed strand in all mouse and human collections of somatically mutated *V(D)J* sequences [38], and these mutations are allowed to propagate due to the DNA polymerase-eta an "error-prone" DNA polymerase. In the DNA repair and replication model, repair has to be precise; however, in some situations an error can cause complete stall in replication, which may be very dangerous to the individual organism. In these cases,

to copy a DNA with some error may help in survival [39]. An error-prone DNA polymerase (polymerase-eta) specifically in this context of SHM can propagate mutations generated in the non-transcribed strand and therefore help in SHM [38, 40].

Additional explanation of increased mutations in the antibody binding site comes from understanding the nucleotide composition of these antigen-binding sites. The variable region is responsible for antigen recognition and contains the antigen-binding sites called the complementarity-determining regions (CDRs) with intervening framework regions (FR regions). Chang and Casali [41] in their study showed that the CDR and FR sequences differ significantly in their inherent susceptibility to amino acid replacement as compared to the result of random nucleotide changes [42]. For every codon representing an amino acid, there are total of nine possible single-base substitutions for its three nucleotides, and a viable substitution results in a replacement (R) amino acid, and a non-viable combination such as a stop codon results in a silent (S) mutation. For example, as cited by the authors, in the codon AGC that codes for "Ser," a single-nucleotide change in any of the three positions (therefore total nine possible), there are eight possible amino acids (Cys, Arg, Gly, Ile, THr, Asn, Arg) and one stop codon. Another example ATG coding for Met could result in nine possible amino acids, and CTG could result in five possible amino acids (Fig. 1.3). They noted that the CDR sequences of all the Ig V_H genes analyzed contain a higher frequency of codons susceptible to replacement mutations than would be expected for a random sequence. Further, the FR sequences comprise codons less susceptible to replacement mutations than expected for a random sequence. So random accumulations of nucleotide changes in CDRs yield a higher rate of amino acid replacements than in the FRs and conceptually make sense as these are under pressure to mutate to provide the best fit for antigen, while the FR sequences need to be preserved in structure in order to provide the scaffolding for the antigen-contacting CDRs. What we need to understand is that this mechanism of mutations is a mechanism of cancer. This is indeed a tightly controlled pathway where mutations help in creating diversity and immune protection.

Microenvironment in the Bone Marrow and Germinal Centers The development of normal B cells from early progenitors requires their interaction with their microenvironment including the stromal cells, T cells, and chemokines along with their unique transcription factors. The development of B-cell precursors through the various stages, termed pro-B cells, pre-B cells, B cells (at which point they exit the BM environment), requires the presence of secreted factors in bone marrow niches such as CXC-chemokine ligand 12 (CXCL12), FLT3 ligand(FLT3L), (IL-7), stem-cell factor (SCF), and receptor activator of nuclear factor B ligand (RANKL), as well as the coordinated expression of specific transcription factors, such as Ikaros, transcription factor E2A, early B-cell factor (EBF), and PAX5 [43]. The chemokines play an essential role in maintaining the quiescent HSC pool and the niches where HSCs reside. These niches are close to the marrow vasculature or to the endosteum. CXCL12 (or stromal cell-derived factor 1 (SDF1)) belongs to a large family of structurally related chemo-attractive cytokines and is a growth-stimulating

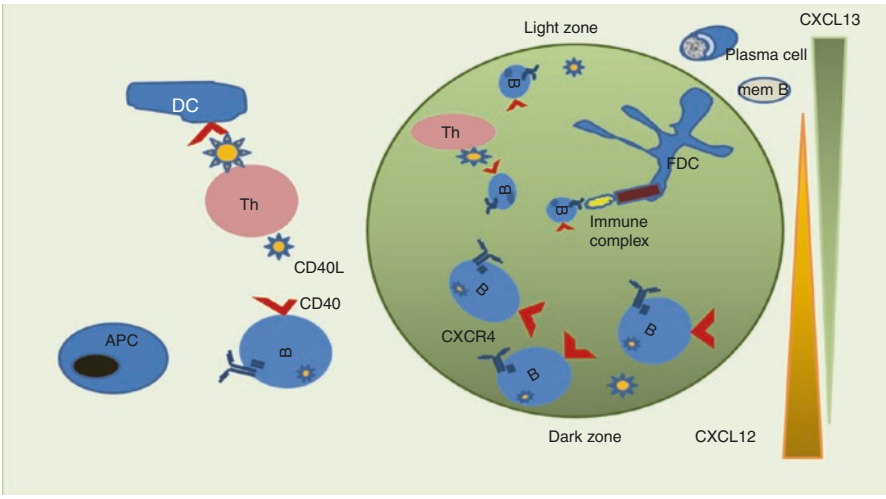

Fig. 1.3 CD40–CD40l interactions play a significant role in germinal center formation. (Adapted from Wykes M et al. [51] and Gatto et al. [59])

factor for the B-cell precursor clone. The primary physiologic receptor for CXCL12 is CXCR4, a heptahelical receptor coupled to heterotrimeric guanosine triphosphate (GTP) binding proteins. CXCL12 and CXCR4 are essential for B-cell development and colonization of bone marrow by hematopoietic cells [44]. FLT3L (also known as FLK2 ligand) is a ligand for FLT3 (also known as FLK2), which has sequence and structural homology to the class-III-receptor tyrosine kinases that include the macrophage colony-stimulating factor (M-CSF) receptor (c-fms). These are important in maintaining and development of immature B-cell precursors [45]. IL-7 and its receptor IL-7R are involved in the B-cell differentiation and induce the expression of myeloid-cell leukemia sequence 1(MCL1) to mediate the survival of B-cell precursors and also induce the recombination of D-heavy chain to distal V-heavy chain segments in pro-B cells [46]. The transmembrane protein RANKL is a tumor necrosis factor (TNF) family member that is essential for the development of osteoclasts and for bone remodeling [47]. All the factors required for B-cell development in the bone marrow are provided by the cells located in the bone marrow and deliver to the appropriate B-cell precursor at the appropriate stage of development. The cells in the bone marrow that create the specific micro environmental niches are the osteoblasts, the stromal/reticular cells that produce high level of CXCL12, IL-7-expressing cells (Fig. 1.3)

Mature B cells migrate to secondary lymphoid organs where they are exposed to antigen within the germinal centers (GCs) of secondary lymphoid follicles. Here B cells regulate their chemokine receptors CXCR5, CCR7, and CXCR4 in order to successfully transit the GC while undergoing the centroblast and centrocyte stages to finally evolve into memory B cells or plasma cells. Their respective ligands CXCL13, CCL19/21, and CXCL12 are secreted by the stromal cells. The light

zones are enriched with FDCs that function as antigen reservoirs for selection of high-affinity GC B-cell clones. FDC are intimately associated with the GC B cells and provide important signals for GC B-cell survival and select those that will become memory cells, otherwise the B cells undergo programmed cell death. They present the major antigen-trapping mechanism of the immune system, which binds antigen in the form of immune complexes for long periods of time and presents these complexes either directly or in the form of immune complex-coated vesicles (iccosomes) to the B cells. B cells adherent to FDC remain viable. FDC express high levels of the adhesion receptors intercellular adhesion molecule 1 (ICAM-1 (CD54)) and vascular cell adhesion molecule 1 (VCAM-1), while the B lymphocytes express lymphocyte function-associated antigen 1 (LFA-1 (CD11a/18)), very late antigen 4 (VLA-4 (CD49d)), and CD44. Both the LFA-1/ICAM-1 and VLA-4/VCAM-1 adhesion pathways are involved in FDC-B lymphocyte binding and are essential in affinity selection of B cells with subsequent formation of B memory cells. Koopman et al. showed that disruption of B-cell-FDC clustering by addition of monoclonal antibodies against CDla, CD49d, CD54, or VCAM-1 (CD106) results in apoptosis of the B cells [48, 49]. Also present are follicular T helper (TFH) cells that provide important signals for GC B-cell survival. CD40, a member of the tumor necrosis factor receptor family, is expressed by B cells, antigen-presenting cells and induces B-cell growth and differentiation. CD40L (CD154) is produced by T cells, antigen-presenting cells granulocytes. B cells also express CD40L on their surface following activation and can release a soluble form of the ligand. B-cell-derived CD40L plays an autocrine role in B-cell proliferation and GC formation. The latter explains the continued GC growth in an environment where T cells account for only 5–10% of the GC and predominantly located in the light zone and perhaps lack the physical ability for the cell-to-cell contact with the large number of B cells in the dark zone [50, 51]. The CD40–CD40L (CD154) interaction is crucial to the development of T-dependent immune responses and provides signals to B cells for B-cell proliferation, immunoglobulin switching, antibody secretion, and rescue from apoptosis at different times during the life of a B cell and a significant role in the development of germinal centers and survival of memory B cells [51–55]. B cells proliferate and acquire high rates of mutations in their immunoglobulin variable region (IgV) genes through the process of somatic hypermutation (SHM). The B cells either traverse the centroblast to centrocyte journey as sequential events described as “cyclic reentry model” or undergo alternating proliferation-dependent migration between the light and dark zones. This analysis indicated that GC B cells in both the light and dark zones are highly mobile and morphologically similar, exhibiting irregular, constantly changing shapes. Cytokine signals also play a central role in triggering the molecular events that lead to the onset of immunoglobulin class-switch recombination (CSR) and thus the production of IgG, IgE, and IgA expressing B cells.

The microenvironment of GC allows maturing B cells to interact with $CD4^+$ T cells for the necessary help on Ag recognition and with specialized follicular dendritic cells (FDCs), for the required quality control after affinity maturation. The antigen encounter triggers the proliferation, maturation, and final differentiation

into effector plasma cells (PCs) and memory B cells [21, 56–60]. The GC reaction and the differentiation of B cells into PCs and memory B cells are regulated by a network of tightly regulated transcription factors that are up- or downregulated during different stages of the naïve B cell, centroblast, centrocyte, and finally to a memory B cell or plasma cell. These include B-cell lymphoma (BCL)-6, PAX5 – a gene considered specific for B cells, B lymphocyte-induced maturation protein (BLIMP)-1, X-box binding protein 1 (XBP-1), interferon regulatory factor (IRF)-4, and signal transducer and activator of transcription (STAT) factors STAT1-5 [61]. The *BCL6* gene encodes B-cell lymphoma 6 protein, which is expressed in GC B cells and is considered as the master regulator of normal GC formation. Downregulation of *BCL6* is necessary for B-cell terminal differentiation. Upon antigen stimulation, TFH cells upregulate the expression of CD40L, which binds CD40 on B cells. This engagement activates nuclear factor-κB (NF- κB) canonical pathways and induces expression of the transcription factor interferon regulatory factor 4 (IRF4) in B cells. IRF4 in turn downregulates the expression of BCL6, enabling terminal differentiation of GC B cells to post-GC lymphocytes. GC B cells are intrinsically prone to apoptosis because of their pro-apoptotic program of gene expression characterized by low levels of BCL2 and BCL-XL and high levels of BIM and Fas (CD95). Therefore GC B cells require antigen-mediated survival signals, and should they not receive these survival signals, it leads to their apoptosis in situ [59] (Fig. 1.4).

BCR Signaling Signals received through B-cell surface receptors are transferred to the nucleus by a cascade of interacting molecules whose structures are temporarily modified during the process. These modifications frequently involve the attachment of phosphate groups to tyrosines of target proteins by specific enzymes. Engagement of the BCR by Ag leads to the phosphorylation of ITAM (immunoreceptor tyrosine-based activation motif) present in the BCR-associated Iga- and Igb-chains [62], by Src family kinases (SFKs) and spleen tyrosine kinase (Syk) [63], and results in recruitment of the *signalosome* [64]. This is a complex of kinases and scaffold proteins tethered at the plasma membrane at sites of sIg activation. The

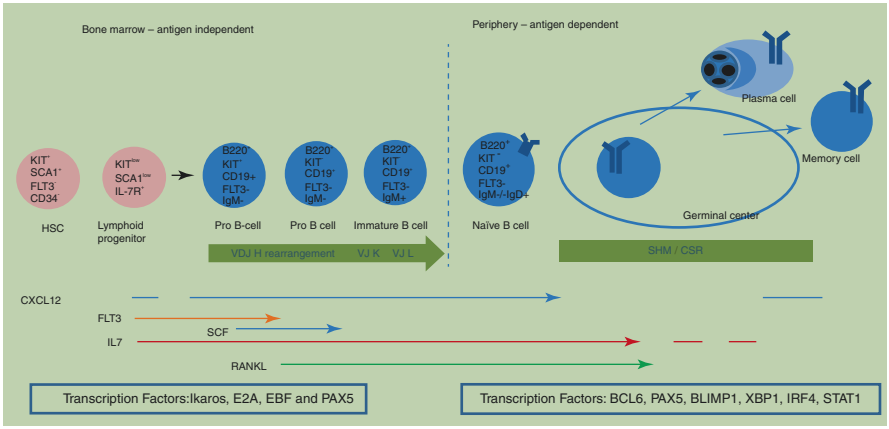

Fig. 1.4 Microenvironment niche for B-cell development. (Adapted from Takashi Nagasawa [43]. In the periphery only the antigen-dependent pathway is shown)

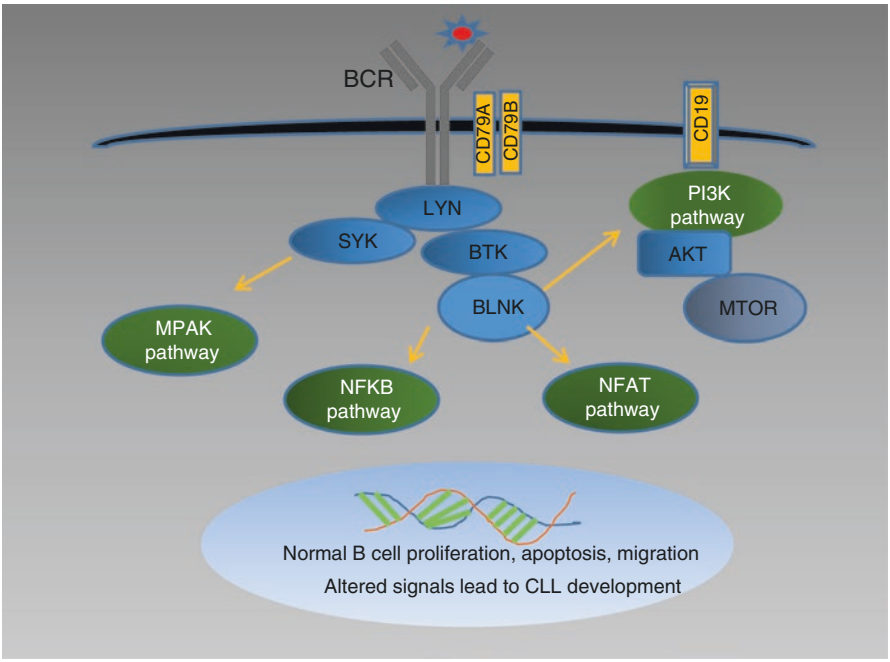

Fig. 1.5 BCR signaling

signal transduction cascade that ensues activates a number of *effector pathways*, such as the P13K pathway [65]; the mitogen-activated protein kinase (MAPK) pathway [66, 67]; nuclear factor-κB (NF-κB) [68], which permit many distinct outcomes, including survival, tolerance (anergy) or apoptosis, proliferation, and differentiation into antibody-producing cells; or memory B cells depending on balancing of the signaling cascades (Fig. 1.5).

Pathobiology of Follicular Lymphoma

FL cells resemble both morphologically and functionally, with normal germinal center B cells. The tumor cells proliferate in networks of follicular dendritic cells and are capable of somatic hypermutation and isotype switching. The variable heavy VH chain genes of FL samples are heavily mutated, and, in addition, isotype-switched lymphomas contain more somatic mutations than immunoglobulin M-positive lymphomas [69]. The lymphoma cells also demonstrate ongoing AID activity, like the normal germinal center cells undergoing SHM and CSR [70].

FL follows a complex multistep process of lymphomagenesis that occurs through several stages of B-cell differentiation. It can be broadly be classified under three successive steps: (a) initiating genetic event t(14; 18) with enforced ectopic

expression of BCL2 in GC-activated B cells leading to their inappropriate rescue from cell death; (b) maturation arrest and accumulation as centrocyte-/centroblast-like cells with ongoing AID activity; and (c) accumulation of oncogenic hits, partly because of AID-mediated genomic instability [11] (Figs. 1.6 and 1.7).

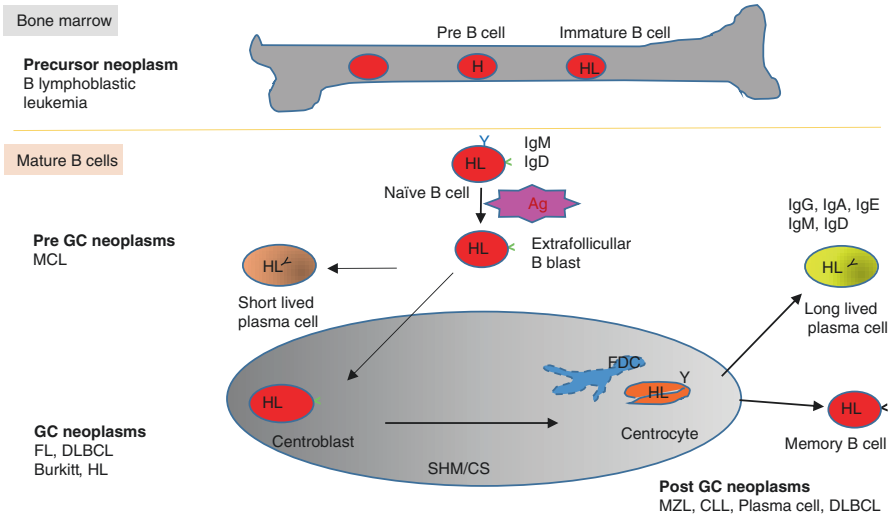

Fig. 1.6 Normal B-cell development and relationship to select B-cell neoplasms

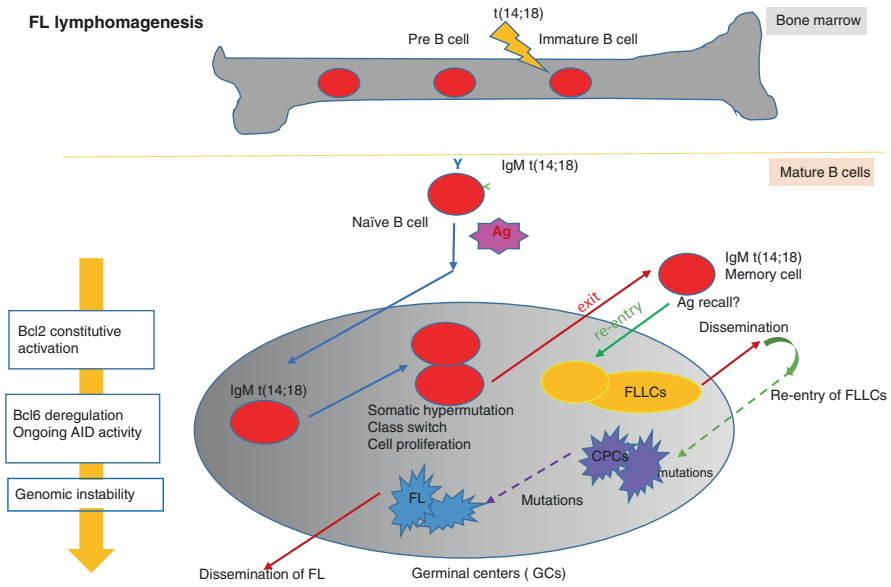

Fig. 1.7 FL lymphomagenesis

The initiating genetic event found in 90% of FL is the t(14;18), between the immunoglobulin heavy chain (IGH) gene promoter regions and the B-cell lymphoma/leukemia 2 (BCL2) proto-oncogene, and the transcripts produced consist of the 5′ half of the Bcl2 mRNA fused to a "decapitated" immunoglobulin heavy chain mRNA [71]. The t(14; 18)(q32; q21) translocation essentially results from a mistake of V (D) J recombination and juxtaposes the *BCL2* proto-oncogene with the *non-expressed* immunoglobulin heavy chain (IGH) allele. By consequence, the translocated genes become regulated by the powerful IgH enhancer leading to the constitutive expression of the anti-apoptotic BCL2. This translocation is acquired in the bone marrow during Ig heavy chain rearrangement, at the time of attempted DH to JH rearrangement, the earliest stage of Ig gene joining in a pre-B cell. This was illustrated when it was seen that the Ig diversity (DH) segments are present at the juncture with 18q21 at the der(18) breakpoint, while Ig joining (JH) segments fused with 18q21 at the der(14) breakpoint [72]. Most breakpoints on chromosome 18 cluster at two sites: in a 150-bp region in the 3′ noncoding portion of the third exon of the *BCL2* gene, labeled as the major breakpoint region (MBR), and the 20- to 30-kb downstream region, known as the minor cluster region (mcr). Breakpoints are also seen on chromosome 18 located between MBR and mcr, referred to as the intermediate cluster region (icr). Occasional additional breakpoint clusters seen are referred to as 3′ BCL2 and 5′ mcr [73–76] (Fig. 1.8).

The t(14; 18) enforced ectopic expression of BCL2 in GC-activated B cells, leads to inappropriate rescue from cell death [77]. Under physiological conditions, B cells lack BCL2 and undergo apoptosis if out-competed during somatic hypermutation and selection. Given their abnormal expression of BCL2, B cells with t(14;18) can escape the normal apoptotic process allowing for prolonged life spans and acquisition of additional oncogenic events. Nucleotide sequence analyses further confirm that the hybrid transcripts continue to encode a normal Bcl2 protein. The t(14;18) translocation alters the expression of the Bcl2 gene both by transcriptional activation and by abnormal posttranscriptional regulation of Bcl2 mRNA [71]. The Bc12 gene in a normal lymphocyte is regulated in a nearly inverse fashion compared with Ig. Bcl2 mRNA is high in pre-B cells but downregulated with differentiation, while Ig mRNA increases with terminal differentiation to a plasma cell. The introduction of Bc12 into the Ig locus results in a deregulation that manifests as inappropriately high levels of Bcl2-Ig fusion transcripts at a mature B-cell stage of development.

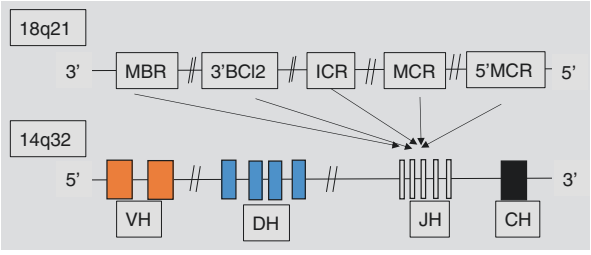

Fig. 1.8 Breakpoint sites in the Bcl2 gene

It is well established that t(14;18) is not sufficient for tumor progression, as B cells bearing this translocation are also detected at very low levels in the blood from 50% to 70% of healthy individuals who will never develop FL, at an average rate of 0.1–10 cells per million [78]. The frequency of cells carrying the translocation appears to increase with age, smoking, and pesticide exposure, and such cells may be less common in ethnic groups with a lower incidence of FL [78, 79]. A subset of these circulating t(14;18)-bearing cells forms an expanding clonal population of atypical B cells. Contrary to what appears logical that these would be naïve B cells, Roulland et al. show that these cells exhibit memory B-cell phenotype and demonstrate CSR. Further, despite CSR in more than 80% of cases, most FL cells express IgM, and only a minority expresses IgG, IgA, or no Ig. This is "allele paradox" when there is simultaneous presence of $sIgM^+D^+$ on the productive allele and of CSR on the nonfunctional allele. In this scenario, despite evidence for class-switch recombination, a functional surface immunoglobulin M (IgM) is expressed and sustains cell survival signals and indicates the presence of selective pressure in favor of IgM expression on the t(14;18)-positive B-cell population that is at the same time permanently driven to switch. It is important to note that the presence of this allelic paradox is in sharp contrast with the features of the peripheral memory B-cell ($IgM^+IgD^+CD27^+$ B cell) subset that are usually devoid of CSR both on the productive and the nonproductive alleles. The t(14;18)-bearing cells retain GC functionality via expression of proteins such as B-cell lymphoma 6 (BCL6) and activation-induced cytidine deaminase (AID) and have been termed follicular lymphoma-like cells (FLLCs) [80, 81]. They display genotypic and phenotypic features of FL and are prone to constitute potent premalignant FL niches [82–84]. In mice models, BCL2-overexpressing B cells require multiple GC transits before acquiring FL-associated developmental arrest and progression to advanced precursor stages of FL [85]. Similar paradigm exists in humans. During this process, further genetic alterations may be added by the effect of activation-induced cytidine deaminase (AID), a DNA editing enzyme that is responsible for both CSR and somatic hypermutation (SHM). These two mechanisms confer a high propensity for further oncogenic aberrations in the context of accumulating genomic instability (e.g., BCL6/p53) [86]. At some point during these multiple GC transits, these FLLCs commit to being a committed FL cancer precursor cell (CPCs).

Microenvironment Manipulation, Epigenetics, and BCR Promote Growth of FL Cells Follicular lymphoma precursor cells or committed FL cancer precursor cells (CPCs) occupy the germinal centers and in this microenvironment, with multiple GC re-entries, develop to full-fledged follicular lymphoma. The molecular signals are similar to developing centroblasts and centrocytes in a normal germinal center. This is not surprising as the germinal center (GC) B cells manifest many hallmarks of cancer cells. The microenvironment includes immunocompetent lymphocytes, stromal cells, and the extracellular matrix. FL cells, like normal GC cells, express the transmembrane receptor CD40, a member of the tumor necrosis factor receptor (TNFR) family that induces B-cell growth and differentiation; its ligand, CD40L, is expressed on T_{FH}. Chemokines such as CXC chemokine ligand 12

(CXCL12) and CXCL13 that regulate growth are secreted by stromal cell subsets, FDCs, and bind to CXCR5 that are present both on T_{FH} cells and FL cells. The CD40 signaling in FL cells leads to increased secretion of IL-4 that in turn activates STAT6-mediated signaling in FL cells, enhancing cell survival. In addition, IL-4 increase the secretion of CXCL12, which regulates B-cell trafficking between the dark and light zones of the normal GC and increases recruitment, homing, and migration of FL cells. PD-1 is expressed on both dysfunctional CD4+ and CD8+ T cells and on fully functional TFH cells. PD-1+ TFH cells co-expressing CD10 secrete high amounts of IL-4, IL-21, and TNF, thereby stimulating the growth of FL cells. Tumor cells evade the antitumor immune response via signals from a variety of cells such as TFH cells, cytotoxic T lymphocytes, and macrophages. The microenvironment also promotes immune evasion mechanisms by the lymphoma cells by reducing tumor immunogenicity, inhibition of immune effectors, and infiltration by immunosuppressive cells [10, 81, 87–89].

An important feature of FL is that, despite the loss of one Ig allele by the t(14;18) translocation, surface immunoglobulin (sIg) is retained, suggesting that a signal provided through the B-cell receptor (BCR) itself and/or its downstream signaling pathway is critical for cell survival. Selective maintenance of a surface IgM in most FL cases might be explained by the fact that IgM signaling promotes the survival and proliferation of B cells, whereas an IgG BCR signaling favors plasmacytic differentiation [90]. It is seen that IgM (+) FL B cells activate a stronger BCR signaling network than IgG (+) FL B cells and normal GC B cells. This results in sustained stimulation of the BCR and its signaling pathways such as NF-κB, MAPK, and PI3K–AKT, which promote proliferation and survival of both normal GCs and malignant B cells. BCL2+ memory B cells support multiple iterative GC entries upon repeated antigen challenge, which promotes AID-induced mutations in a mutagenic environment and, in turn, propagates clonal evolution toward FL progression [85].

Antigen-independent BCR signaling is seen due to somatic mutagenesis in the variable regions of immunoglobulin heavy and light chain genes. In FL, variable Ig regions are frequently N glycosylated (80% compared to 10% in normal B cells). The introduction of N-glycosylation sites occurs during somatic hypermutation, and these acceptor sites harbor unusual high-mannose oligosaccharides. The carbohydrate modifications modulate interactions with surface structures (C-type lectins, dendritic cell-specific ICAM-grabbing non-integrin (DC-SIGN), and mannose receptors) present on other cells such as macrophages and FDCs. Additionally the immunoglobulins on FL cells that bind DC-SIGN are not internalized and stay on the membrane, creating a persistent signal [91]. Increased BCR signaling FL was confirmed by demonstrating sustained activation of BCR-associated proteins such as BTK, SYK, and ERK in lymph node biopsies. It is seen that pharmacologic BCR inhibitors abolished such crosstalk between macrophages and FL B cells [92–94].

EpigeneticDysregulationProvideAdditionalSurvivalPathways [81] Epigenetic modifications involve addition and removal of chemical groups to DNA or histones. Follicular lymphoma depends on deregulated epigenetic control of gene

expression. Genetic alterations in chromatin-modifying genes that are present alone or in combination include mutations in the H3K4 histone methyltransferase KMT2D (also known as MLL2). These are the most frequent abnormality, found in 80–90% of patients with FL. Mutations in enhancer of zeste homologue 2 (EZH2), the catalytic subunit of the polycomb PRC2 complex involved in histone methylation, are found in >25% of cases. Mutant *EZH2Y641* with reduced MHC expression favor tumor progression with acquired immune escape. Deleterious and/or loss of function mutations in the histone acetyltransferase *CREB binding protein (CREBBP)* or the *E1A binding protein 300 (EP330)* have been reported in about 40–70% of FL patients. Recurrent point mutations in the histone acetyl transferase (HAT) recruiting gene *myocyte enhancer binding factor 2B (MEF2B)* have been also described in 15% of FL. CREBBP mutations have been shown to facilitate immune evasion by downregulating major histocompatibility complex (MHC) class II expression in FL that alter and decrease helper and cytotoxic T-cell subset in FL. One of the commonly affected regions is chromosome 1p35, which contains TNFRSF14. Copy number alterations, acquired copy neutral loss of heterozygosity, and mutations in TNFRSF14 are common in all forms of FL, including the diffuse variant and pediatric-type FL. Mutation in HVEM (herpesvirus entry mediator; TNFRSF14) receptor gene leads to cell-autonomous activation of B-cell proliferation and drives the development of FL. HVEM-deficient lymphoma B cells also induce a tumor-supportive microenvironment marked by exacerbated lymphoid stroma activation and increased recruitment of T follicular helper (T_{FH}) cells. These changes result from the disruption of inhibitory cell-cell interactions between the HVEM and BTLA (B and T lymphocyte attenuator) receptors [95]. HVEM deficiency also induced the secretion of activating cytokines (tumor necrosis factor (TNF), lymphotoxin-α (LTα), and LTβ), which create a tumor-supportive environment containing more follicular helper T lymphocytes secreting interleukin-4 (IL-4) and IL-21. CD19-targeted chimeric antigen receptor (CAR) T cells that produce soluble HVEM locally and continuously have shown enhanced therapeutic activity against xenografted lymphomas and illustrate use of CAR–T cells as "micro-pharmacies" that deliver anti-cancer proteins. Overall, these epigenetic alterations shift the equilibrium away from transcriptional activation toward the aberrant repression of gene transcription. The epigenetic dysregulation likely cooperates to freeze the functional states of GC B cells, which are characterized by intense proliferation and genomic instability, thus favoring their malignant transformation toward FL [81, 96, 97]. Proof of concept is shown by histone deacetylase 3 (HDAC3)-selective inhibitors that fully reverse mutant *CREBBP* aberrant epigenetic programming, resulting in the restoration of immune surveillance due to induction of the interferon pathway and antigen presentation genes. EZH2 inhibitors restore MHC expression in *EZH2*-mutant lymphoma cell lines [98, 99].

B cells "arrested" in germinal centers acquire dozens of *additional genetic aberrations* that influence key pathways controlling their physiological development including B-cell receptor (BCR) signaling, Bcl6, PI3K/AKT, TLR, mTOR, NF-κB, JAK/STAT, MAPK, CD40/CD40L, chemokine, and interleukin signaling. A small

fraction of FL (15%) does not exhibit the classical t(14;18) but instead contains alterations affecting BCL-6 at 3q27, including t(3;14) (q27;q32). This leads to deregulated expression of the transcriptional repressor BCL-6, normally required for GC formation. These FLs most often display an exclusive centroblastic morphology, may be BCL2 negative, and are classified by the WHO as grade 3B. Translocation involving *BCL6* – most often t(3;14) (q27;q32)/*BCL6–IGH* – have been identified in 10–15% of cases of FL. Somatic mutations of *BCL6* also occur commonly. BCL6 transcription factor plays a central role in establishing the GC phenotype in B cells, and FL too is dependent on BCL6 to maintain survival, proliferation, and perhaps immune evasion. Mutations in *STAT6* have been reported in >10% of FL cases [97, 100–104]. Table 1.1

Cytogenetic Abnormalities In addition to t(14;18), additional genetic abnormalities seen in 90% of FL include loss of 1p, 6q, 10q, and 17p and gains in chromosome 1, 6p, 7, 8 12q, X, and 18q [105] [WHO 2016].

The molecular analysis of the tumor at different time points of disease progression has demonstrated that *lymphomagenesis* follows a complex multi-hit process. The multistep progression of follicular lymphoma is demonstrated morphologically as a continuum from in situ follicular neoplasia(where aberrant CD10 + Bcl2+ cells colonize germinal centers), duodenal FL (indolent FL that occupies the mucosa/submucosa and the neoplastic B cells infiltrate outside the follicles), and partial involvement by FL(true signs of lymphoma adjacent to reactive germinal centers) (Fig. 1.10). The clinical course, however, likely represents the tip of the iceberg, as a large part of this very complex process might take years, if not decades, before diagnosis [106]. The long preclinical phase is illustrated dramatically, by the donor–recipient pair, who synchronously developed FL (grade 2/3a) 9 and 7 years after allogeneic transplantation and donor lymphocyte infusion, respectively. Both donor and recipient harbored the same malignant FL clone, with over 90% of shared mutations, identical Bcl2/IGH rearrangements, and the same V(D)J rearrangement. Six additional mutations were present in only one follicular lymphoma and not the donor lymphocyte infusion, indicating later acquisition during clonal divergence, demonstrating acquisition at least 7 years before clinical presentation. This report also revealed the capacity of the precursor cells to develop once transplanted in an allogeneic host. The study provided direct proof of principle that a "committed" FL precursor can be present in the bone marrow (BM) and/or blood, long before diagnosis [107].

Clinical Features/Staging

FL is generally a systemic disease, involving lymph nodes, spleen, Waldeyer's ring, bone marrow, and blood. Secondary involvement of "extra nodal" sites such as the gastrointestinal tract, soft tissue, and skin may occur at advanced stages. FL is occasionally found primarily at extra nodal sites such as the skin, ocular adnexa,

Table 1.1 Recurrent genetic alterations in FL

Recurrent genetic alterations in FL				
Pathway	Gene	Function	Oncogenic alteration	Frequency (%)
BCR signaling	IgH, IgL variable domains	BCR signaling, promotes glycosylation	Gain of function	80
	CARD11	BCR–NF-κB signaling pathway	Gain of function	10–15
	FOXO1	Transcription factor activated downstream of BCR signaling	Gain of function	10
Epigenetic and transcriptional regulation				
	KMT2D	Histone H3K4 methyltransferase	Loss of function	70–90
	CREBBP	Histone H3K27 and H3K18 acetyltransferase	Loss of function	50–70
	EZH2	Histone H3K27 methyltransferase	Gain of function	10–30
	Bcl6	Transcriptional inhibitor	Gain of function	Mutations, ~5; translocations, ~10
Survival				
	Bcl2	Anti-apoptosis	Gain of function	Translocations, ~85 Mutations, ~50
	SOCS1, *STAT6* and *STAT3*	JAK–STAT signaling	Gain of function	20
	NOTCH1, *NOTCH2*, *NOTCH3*, *NOTCH4*, *DTX1*, and *SPEN*	NOTCH pathway	Unknown	18
Immune escape				
	HVEM	Receptor	Loss of function	~50%
	EPHA7	Ephrin receptor/tumor suppressor	Loss of function	70%
mTORC1 signaling				
	RRAGC	Guanine nucleotide-binding protein	Gain of function	10–15

gastrointestinal tract, and the female genital tract. The presenting signs consist of enlargement of lymph nodes in the neck or abdomen. Enlarged lymph nodes of the abdomen are typically found incidentally on imaging. The symptoms include fatigue, fever or night sweats, weight loss, or recurrent infections. Most may have no obvious symptoms of the disease at the time of diagnosis. Extra nodal FL can cause a variety of symptoms depending on its location, for example, patients with bone marrow disease might develop anemia, thrombocytopenia, and/or neutropenia. Initial diagnosis is preferably rendered on excised lymph nodes. The emphasis is placed on excised lymph nodes as opposed to needle core biopsies. This is optimal for delineation of different morphologic subtypes, separating from other low-grade lymphomas, grading, and ruling out transformation to large cell/high-grade lymphoma. Needle core biopsies or fine-needle aspiration can be performed in the elderly but may be limiting for differentiating all the lesions already mentioned. Flow analysis is helpful in delineating clonality but should not be used as a substitute for a complete morphologic evaluation. Bone marrow biopsies are obtained for staging purposes. Blood tests to evaluate blood cell count, measure lactate dehydrogenase levels, and screen for viruses such as HIV, hepatitis B virus and hepatitis C virus, and imaging (CT and PET) are routinely performed as a part of staging to plan the treatment [108].

Microscopy

The cytological and architectural features of FL are highly reminiscent of those of normal germinal centers (GC). The tumor cells resemble normal centroblasts and centrocytes that proliferate in follicles in a network of non-malignant follicular dendritic cells (FDC) and T cells. The neoplastic cells are small- or medium-sized centrocytes with irregular cleaved nuclear profiles and mature clumped nuclear chromatin and large transformed centroblasts cells that contain multiple prominent eosinophilic peripheral nucleoli that may touch the nuclear membrane. Sometimes centroblasts have irregular or multilobed nuclei. The morphology of the centroblasts is determined by the nuclear characteristics as these cells can be both large and small. In rare cases, the lymphoma is composed of blastoid-appearing cells with fine dispersed chromatin resembling lymphoblasts. They behave aggressively and are considered equivalent to grade 3 [109]. The interfollicular component of FL usually contains the low-grade centrocyte population, showing a low proliferative index and downregulation of activation markers such as CD10, CD38, and CD95 and T-cell costimulatory molecules CD80 and CD86; markers normally expressed by neoplastic follicles. The low-grade morphology and immunophenotype suggest that these are resting cells and analogous to memory B cells of normal lymphoid tissues. The presence of such a resting tumor cell subpopulation in the majority of follicular lymphomas may partly account for the remarkable resistance to therapy of this disease [110]. The lymphoma cells are admixed with different numbers of reactive T cells, follicular dendritic cells (FDCs), histiocytes, granulocytes, and plasma

cells. Some cases can show plasmacytic differentiation, with plasma cells showing similar light chain restriction as the neoplastic follicular lymphoma cells and t(14;18) [111].

Bone marrow studies are performed for staging. Bone marrow involvement is equivalent to stage IV disease. FL characteristically involves the paratrabecular regions, and the lymphoid aggregates may be associated with reticulin fibrosis. These aggregates are difficult to aspirate, and therefore the aspirate specimens interrogated by flow analysis may not show the aberrant population. Sometimes the abnormal lymphoid infiltrates are best delineated by IHC stains for CD20. The bone marrow neoplastic aggregates may lack staining with CD10 (due to altered BM cytokine milieu) but can be highlighted with Bcl6 [110].

Immunophenotype

The tumor cells express B-cell markers such as CD19, CD20, CD22, and CD79a; the GC B-cell markers BCL-6, CD38, and CD10; and membrane-bound immunoglobulin (mIgM >mIgG >mIgA). Ninety percent of FLs express the anti-apoptotic BCL2 protein owing to the t(14;18). They are negative for CD5 and CD43. Grade 3 FLs may lack CD10 but will express Bcl6 [112]. CD10 can be negative in the interfollicular centrocytes, in BM infiltrates, and in areas of marginal zone differentiation [110]. Likewise, Bcl6 are frequently downregulated in interfollicular areas. Germinal center markers such as LMO2, GCET1, and HGAL are positive [113]. CD5 has been described positive in rare cases of FL, more frequently in the floral growth pattern [114, 115]. Some FLs show CD10-/MUM1+ phenotype [116]. The Ki-67 proliferation index generally correlates with histologic grade. Most grade 1–2 cases have a low proliferation index (PI) <20%, while grade 3 have a PI >20%. The proliferation index is usually assessed in the intrafollicular areas, and both CD20 and CD3 stains should be performed along with KI-67 to ascertain the proliferation fraction contributed by B or T cells. The PI has been a consideration in lieu of grading by centroblast count. Centroblast count is a major problem in histological grading due to considerable intra- and inter-observer variability. However, studies on PI too have not shown reproducible results. One of the robust studies was performed with CD20/mib-1 and CD3/mib-1 immunohistochemical double staining, thus visualizing proliferating B-cells and non-tumor T cells, and more than 1000 cells were counted. This may have limitations in practical application especially in laboratories that may not have the provision for double staining. Perhaps for these reasons, centroblast counts remain the standard for grading FLs, and the use of Ki-67, albeit clinically justified, is not a formal requirement [1](WHO 2017).

BCL2 is negative in a great majority of high-grade FLs. Some cases may show apparent absence of Bcl2 due to mutations in the Bcl2 gene that eliminates the epitopes recognized by the most commonly used antibody [117]. BCL2 can be detected by using antibodies to other Bcl2 epitopes. BCL2 is useful in separating FL from reactive hyperplasia but not from differentiating other low-grade B-cell lymphomas

as most of these are positive for Bcl2. Caution should be exercised while interpreting BCL2 in nodules of small B cells. Nodules of primary follicles by definition are positive for CD20+ and BCL2 and negative for CD10. It needs to be ensured that they are CD20+/CD10+ and or BCL6+ germinal center lymphocytes that are expressing BCL2. Additionally T cells and mantle zone cells are positive for BCL2. Usually germinal center neoplastic B lymphocytes show a weaker expression of BCL2 as compared to BCL2+ normal intrafollicular T cells. B-cell clonality assessment and assessment for BCL2 rearrangements are performed in challenging cases. Clonality assessment by polymerase chain reaction (PCR) tests is associated with a high rate of false negatives, due to ongoing somatic immunoglobulin variable region heavy chain (IgVH) hypermutations in FL. Robust multiplex PCR assays are available that have maximized the detection of the *t(14;18)*. The assays are capable of amplifying across the breakpoint region in the majority of cases of FCL with a cytogenetically defined translocation [118]. Fluorescence in situ hybridization (FISH) is also a preferred method to detect t*(14;18)(q32;q21)* chromosomal translocation. FISH analysis has a greater diagnostic impact by separating follicular lymphoma from reactive follicular hyperplasia and from lymphomas other than follicular lymphoma [119, 120]. This is well demonstrated by MALT lymphomas that show t*(14;18)(q32;q21)*; however the gene involved is not Bcl2 on chromosome 18 but instead is MALT1. On conventional karyotyping, both will show the same translocation.

Grading

FL is graded by counting the absolute number of centroblasts in ten representative neoplastic follicles (not chosen for high centroblast count), expressed per high-power microscopy field (×40 objective, 0.159 mm^2). Grade 1–2 cases have <15 centroblasts/HPF (grade 1: 0–5 centroblasts/HPF; grade 2: 6–15 centroblasts/HPF). Currently, as recommended in the 2008 WHO classification, grade 1–2 are combined as there is no clinically significant difference between grades 1 and 2. Grade 3 cases have >15 centroblasts/HPF. Grade 3 is further subdivided on the basis of the presence or absence of centrocytes. Grade 3A has centrocytes, whereas grade 3B are composed entirely of centroblast or immunoblasts. Immunoblasts are large lymphocytes with ample cytoplasm and prominent single nucleoli. If grade 3 areas are present in a biopsy containing primarily grade 1–2, then a separate diagnosis of grade 3 should be made, and approximate percentage of each grade should be reported (WHO 2017) [1, 106, 121]. Diffuse areas with grade 3 cytology warrant a diagnosis of diffuse large B-cell lymphoma (DLBCL). Grade 3B FL is biologically more closely related to DLBCL [122]. In contrast to other FL, FL3b frequently lack CD10 expression (approximately 50% of cases), show lower probability of BCL2 expression (70% positive), and increased TP53 expression (31% positive). The t(14;18)(q32;q21) hallmark translocation of FL is present in <5% cases of FL3b. In contrast, translocations affecting the BCL6 locus in 3q27 are frequent (>40%).

There may be higher short-term mortality, but patients in remission 5 years post-anthracycline-based therapy are likely cured [123]. Overall, FL3B in many features resembles DLBCL. The presence of a diffuse component in FL3B has been related to an unfavorable outcome. Thus, follicular lymphomas grade 1–3A behave indolent and are incurable with conventional therapy. Grade 3B appears to be an aggressive but curable disease. These however need further follow-up studies [124].

Morphologic Patterns

The lymphoma in most cases has a follicular pattern with back-to-back nodules. The nodules are usually, poorly defined, with attenuated mantles. They lack polarization of centroblasts and centrocytes, which are usually randomly distributed. Tingible body macrophages are usually absent. The follicles may be irregular or serpiginous. The lymphoma often extends into perinodal fat. Spread beyond the node is associated with sclerosis, and this is most often seen in mesenteric and retroperitoneal locations. Four patterns of FL are recognized in histological examinations: the follicular pattern (>75% follicular); follicular and diffuse pattern (25–75% follicular pattern); predominantly diffuse, focally follicular (<25%follicular); and a diffuse pattern (absence of follicular areas) (WHO 2017). Staining for FDC markers (CD21/CD23) can be used to highlight follicular pattern. The lymph node may be partially involved in some cases with residual germinal centers present. Interestingly, these FLs that demonstrate partial involvement of lymph nodes present also with limited clinical stage (I/II) disease [125].

Diffuse follicular variant is a novel FL variant characterized by:

- Presentation mostly in inguinal region.
- Diffuse growth (lack CD21-positive FDC meshworks).
- Absence of t(14;18)(q32;q21) (IgH/Bcl2 translocation).
- Low-grade morphology.
- Phenotype CD10+, Bcl6+, CD23+, Bcl2-.
- Recurrent genetic aberration with deletion in chr 1p36 (that includes the gene TNFRSF14). Cases that lack 1p36 deletion have demonstrated TNFRSF14 mutations highlighting the strong association of 1p36/TNFRSF14 abnormalities with this follicular lymphoma variant.
- The gene expression profiles fall within the spectrum of typical FL.

The neoplastic infiltrate consists of a diffuse infiltrate of centrocytes and centroblasts. Residual germinal centers may be present with preserved mantle zones, but in most cases, they are ill-defined and partially colonized and can be recognized by immunohistochemistry (CD10+, BCL6+, BCL2-, CD21+, and high Ki-67). No significant plasmacytic or monocytoid differentiation is described. These show small so-called micro-follicles with weak to absent Bcl2 staining. The neoplastic B cells are CD20-positive, CD10+, and Bcl6+ and negative for CD5, CD43, Cyclin D1, and

LEF1. CD21 stain follicular dendritic cell networks within the few residual reactive follicles, while the lymphoma is devoid of staining. *CD23* co-expression by the lymphoma cells is described as strong and uniform. The diffuse lymphoma shows variable expression of BCL2 and a lower proliferation index. These present as large tumors, mostly in the inguinal region and tend to remain localized in this area without dissemination. CD10 negativity can raise concern for marginal zone lymphomas, but the morphology, BCl6+, LMO2+, is helpful [126]. Bone marrow involvement includes diffuse and paratrabecular involvement. This follicular lymphoma variant is genetically characterized by a high incidence of mutations in *STAT6* and *CREBBP*, loss or mutation of *TNFRSF14*, reduced frequency of *KMT2D* mutation, *BCL2* mutations, negative for t(14;18)(q32;q21) involving the *IGH/BCL2* rearrangement, and lack of gain of 18q and mutation of *TP53*. Treatment has ranged from RCHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone) or similar chemoimmunotherapy, excision alone, or radiation alone. Recurrences can be seen. Interestingly, CD10+ marginal zone lymphoma has been described, curiously also involving inguinal nodes [127]. This may represent a clinicopathologic gray zone between diffuse follicular lymphoma and marginal zone lymphoma.

"Floral" *follicle center lymphoma (FCL)* is a distinct morphologic variant of FCL first described by Osborne and Butler in 1987 and later eloquently by Tiesinga et al. [115]. The morphology is that of lymphomatous nodules surrounded and infiltrated by small lymphocytes of the follicular mantle, resulting in nodules with an unusual serrated configuration. The nodules show irregular central areas composed of centrocytes and centroblasts surrounded by intact and somewhat broadened mantle zones. The central areas are irregular or ill-defined owing to encroachment of small mantle zone lymphocytes into the periphery of the germinal center. Infiltrating mantle cells can be differentiated from centrocytes. The centrocytes are larger (1.5×), show greater nuclear irregularity in the form of clefting, and have a more vesicular, less hyperchromatic chromatin. The process can blur the germinal center–mantle zone interface and can impart an appearance resembling progressive transformation of germinal centers (PTGC), or can be confused with lymphocyte dominant Hodgkin lymphoma. These lymphomas express CD20+, Cd10+, and a significant subset express CD5. All demonstrate Bcl2 rearrangement and are clonal by southern blot or PCR techniques. The CD5 expression can cause confusion with small lymphocytic lymphoma and mantle cell lymphoma. In the study cited here [115], they show that the infiltrating mantle zone cells are CD20+, CD5 negative, while the germinal center neoplastic cells show positivity for CD5. They also demonstrated CD20+/CD5+/CD10+ phenotype of the neoplastic cells by flow analysis (elegant study confirming that the CD5-positive cells were not the infiltrating mantle zone cells). These are graded like regular FL, based on Mann-Berard criteria [115].

FL with *marginal zone differentiation*. In about 10% of FLs, there is marginal zone differentiation or the presence of marginal zone-like cells/monocytoid B cells, at the periphery of the nodules. The neoplastic FL cells acquire the molecular, cytogenetic, and phenotypic characteristics of marginal zone lymphoma (CD10-,

abnormalities in Chr 3; features usually seen in MZL cells) [128–131], and some have alluded to a more aggressive clinical course of these lymphomas.

Testicular Follicular Lymphoma

Testicular FLs are unusual as they present primarily in the pediatric age group. What makes it even more unusual is the clinical presentation. Pediatric testicular tumors are most frequently of germ cell derivation and gonadal stromal neoplasms, rhabdomyosarcomas, and leukemia. Lymphomas are very rare (reviewed in [132]). In childhood, when lymphomas are seen, they usually represent a secondary involvement by an advanced stage Burkitt's or lymphoblastic lymphoma. In general, lymphomas occur in the elderly, with aggressive histology and poor prognosis. Therefore, pediatric primary follicular lymphoma of the testis (PFLT) is an unusual rarity. Pediatric PFLT is seen in prepubertal boys (age range from 3 to 11 years). The cases show effacement of tissue architecture or infiltration between seminiferous tubules in a nodular or follicular pattern of large lymphocytes (centroblasts) with some small cleaved centrocytes. Prominent fibrosis is seen, often involving the capsule.

The biologic features of pediatric PFLT demonstrate some differences from follicular lymphomas in adults. Bcl2 protein is not detected. Molecular analysis has revealed monoclonal immunoglobulin heavy chain gene rearrangement and BCL-6 mutations. These mutations characteristically occur in antigenically stimulated B cells, which enter the germinal center, carry out Ig class switching, rearrange their Ig V region to recognize specific antigenic determinants, and undergo selection based on the affinity of the antibodies produced. They lack BCL2 major breakpoint and BCL2 minor cluster region rearrangements, p53 mutations, and death-associated protein kinase gene hypermethylation [133]. When the disease is limited to the testis, complete resection by unilateral orchiectomy followed by limited chemotherapy (that may include cyclophosphamide, vincristine, prednisone, doxorubicin) without intrathecal chemotherapy has been suggested, with favorable prognosis [134].

Of note, primary NHL of the testis in adults is most frequently DLBCL of the non-germinal center B-cell-like (GCB) group. It is most often localized disease, but survival is poor even with intensive chemotherapy regimens (60% overall survival at 5 years). Recurrent NHL/DLBCL of the adults often involves other extra nodal sites such as the central nervous system (CNS). In pediatric PFLT, the excellent outcome with low intensity treatment of short duration without intrathecal chemotherapy is a distinctive difference from adult primary DLBCL of the testis.

In summary, PFLT in children and adolescents is a unique subset of pediatric NHL. It occurs at an unusual extra nodal site with localized disease limited to the testis. The clinical outcome is excellent with treatment including complete surgical resection and short duration chemotherapy [132].

CD10-/MUM1+ FL FLs are positive for CD10 and negative for IRF4/MUM1 (consistent with the germinal center phenotype). MUM1 (multiple myeloma oncogene 1)/IRF4 (interferon regulatory factor 4) is a lymphoid-specific member of the interferon regulatory factor family of transcription factors, and it is a reliable marker of "late-stage GC" or "post-GC" B cells.

Recently, CD10-negative, MUM1/IRF-positive cases of FL have been described [135, 136]. All $CD10^{-}MUM1^{+}$ FL patients exhibited follicular structure with follicular dendritic meshwork, and a high rate of ongoing somatic hypermutation – *similar to typical FL*. Karube et al. report the frequency of somatic mutation of immunoglobulin heavy chain in $CD10^{-}MUM1^{+}$ FLs ranging from 2.04% to 25.69%, with a mean of 11.81%. Intraclonal diversity, which reflects ongoing mutation, was detected in all patients, suggesting definitive germinal center B-cell origin. These lymphomas present in the elderly, in lymph nodes, show a distinct follicular pattern with CD21+ follicular dendritic meshworks, and some show significant diffuse areas. They tend to show high-grade morphology. They lack Bcl2 translocation and instead show amplification of BCl6. These patients show relatively poor prognosis [116, 137]. Because these lymphomas show a genetic profile that is similar to FL, they are described here. Of note, this entity is described within the "immunophenotype" section of FL in the WHO 2017.

These MUM1 FL should be distinguished from IRF4+/MUM1+ large B-cell lymphoma, which can also present with a follicular growth pattern (yes, we unfortunately thought follicular lymphomas are easy to diagnose). The IRF4+/MUM1+ DLBCL present in the young age group with predominantly involvement of the Waldeyer's ring or head and neck lymph nodes.

In Situ Follicular Neoplasia

In situ follicular neoplasia (ISFN) is defined as colonization of germinal centers by clonal B cells that carry Bcl2 translocation, characteristic of FL, in an otherwise reactive lymph node. The germinal centers may be partially or completely colonized. ISFN can be seen in 2% of randomly selective reactive lymph node biopsies. The ISFN component can be seen in lymph nodes that are otherwise involved by other lymphomas such as marginal zone lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, diffuse large B-cell lymphoma, and classical Hodgkin lymphoma, or lymph nodes dissected for staging other cancers. FL can follow ISFN as late as after 10 years. This was formerly called follicular lymphoma in situ. ISFN is not the same as partial involvement of lymph node by FL. The latter is considered a form of FL (WHO 2017). While partial involvement by FL can be suspected by morphologic evaluation alone, ISFN is not obvious by morphology alone and is detected by staining with CD10 and Bcl2. Bcl2 is often very brightly expressed in comparison to adjacent T and mantle zone cells. The clinical significance of ISFN is variable with some patients presenting with synchronous FL at other sites at

presentation, others developing subsequent lymphoma, usually FL, with further follow-up, and others that may never progress to FL. The risk of subsequent development of FL is low (<5%). There is no prognostic significance associated with the number of follicles involved [138].

The concept of intrafollicular neoplasia/"in situ" lymphoma is properly applied when the neoplastic cells are localized in the "place" that is occupied by the normal counterpart of the tumor cell, without invasion of surrounding structures. This can be seen in lymphoid tissue in nodal and extra nodal sites such as the spleen (WHO 2017). These clonal B cells appear to be at an earlier stage of follicular lymphoma and are similar to FL-like cells circulating in peripheral blood or duodenal follicular lymphoma [139]. FL-like cells and ISFN have been described in the same patient. ISFN, as described earlier, are positive for t(14;18) but with very few additional genetic aberrations [139]. The cases suggest that ISFN may represent either a pre-neoplastic event or an early stage of FL. The detection of ISFN may represent the initial seeding of reactive germinal centers by monoclonal B cells. Subsequent development of FL still warrants additional mutations that result in the expression of malignant behavior [140] (Fig. 1.9).

Duodenal-Type Follicular Lymphoma

Duodenal-type follicular lymphoma (DFL) is a variant of follicular lymphoma with distinctive clinical and biological features. DFL presents most often in the second part of the duodenum, as mucosal warty polyps, discovered incidentally on endoscopy performed for other reasons and behaves as a very indolent disease. DFL-type lesions can be seen in the distal small intestine. The neoplastic follicles are confined to the mucosa and submucosa without dissemination outside the intestinal wall. The neoplastic cells may infiltrate the lamina propria and extend into the villi. The neoplastic follicles show low-grade morphology with mostly centrocytes and few centroblasts. The atypical follicles show strong immunohistochemical expression of CD20, CD10, and BCL2, variable Bcl6, in addition to the t(14;18) translocation. The proliferative index is low. The neoplastic lymphocytes commonly express IgA, rather than IgG or IgM consistent with intestinal origin, CD21+ FDC meshworks can be seen in the periphery of the follicles. This entity shows features intermediate between ISFN and FL. The immunophenotype is similar to ISFN; however, unlike ISFN, in which the cells remain confined to the follicular structure, the cells in DFL spread outside the follicles; and, unlike FL, it is not systemic and does not extend into the intestinal wall, or regional lymph nodes. CGH analysis reveals genomic alterations with similar frequencies to those observed in partial involvement by FL, but only a small fraction of the genomic alterations seen in FL such as mutations in TNFRSF14 gene; amplified oncogenes BCl6, Bcl2, and FGFR1; and deleted tumor suppressor genes such as PTEN, FAS, and TP53. Gene expression studies have shown similarities to MALT lymphoma and are thought to arise from memory B cells with evidence of somatic

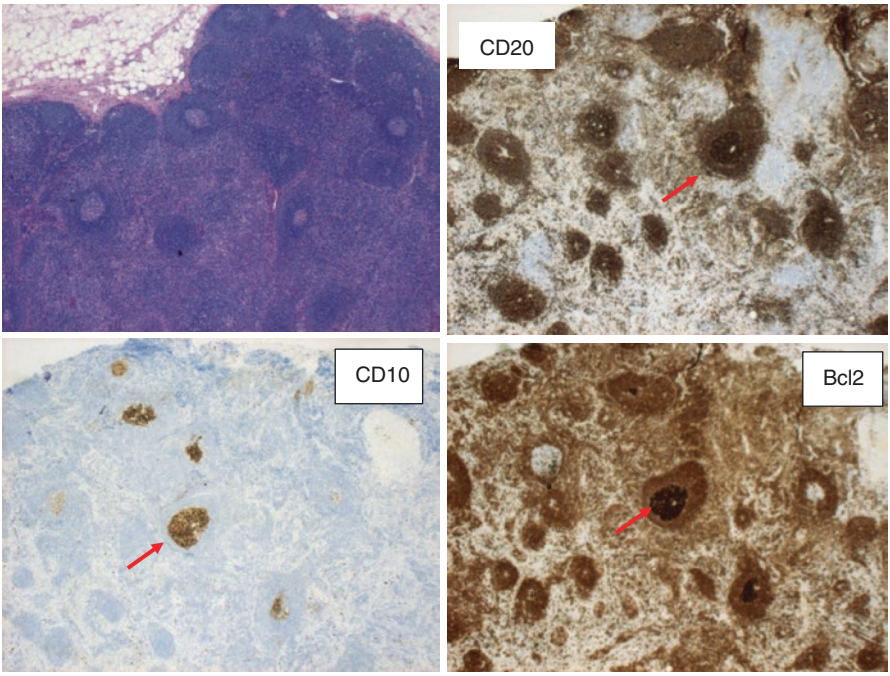

Fig. 1.9 In situ follicular neoplasia: CD20-positive follicles express CD10 and strong positivity for Bcl2

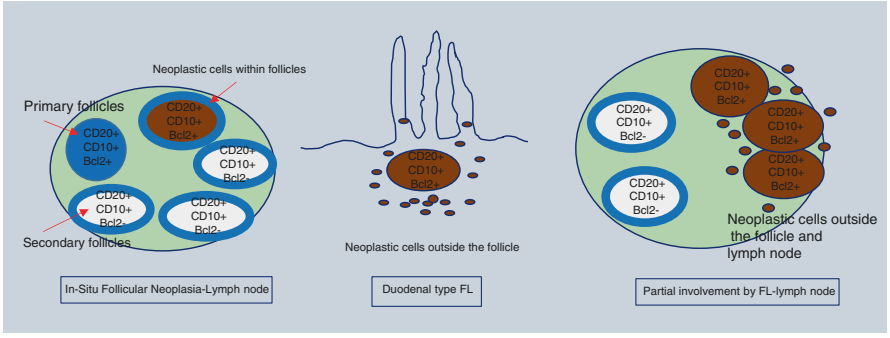

Fig. 1.10 Diagrammatic representation of In-Situ Follicular Neoplasia, Duodenal type FL and partial involvement by FL

hypermutation of the IgH gene. It is an indolent disease, with excellent long-term survival. There is very low rate of progression to nodal disease (<10%). Various therapies have been used such as watch and wait strategy, radiotherapy, anti-CD20 antibody monotherapy, and occasionally chemotherapy. Cases that do not exhibit the typical features of DFL should be evaluated for evidence of FL elsewhere [141–144]. The comparison for in situ FN, duodenal FL, and partial involvement by FL is demonstrated in Fig. 1.10.

Transformation

Transformation or progression to *DLBCL* occurs in 20–30% of the cases, the most common type of transformation. Transformation to *high-grade B-cell lymphoma* occurs, in a minor subset. The high-grade B-cell lymphomas include those that acquire additional MYC genetic abnormalities along with Bcl2 and are called double-hit lymphomas. Some transform to high-grade B-cell lymphomas, NOS that have morphologic feature of blastoid lymphomas, or feature between DLBCL and Burkitt Lymphoma but by definition lack the genetic double-hit abnormalities. Others transformations include *B lymphoblastic leukemia (CD19+/CD20+/CD34+/TdT+)*. These may harbor MYC rearrangements, along with Bcl2 rearrangement, but are not included as double-hit lymphomas. Other rare transformations are *Hodgkin lymphoma* and *histiocytic or dendritic* cell sarcoma. Transformation can be proved by demonstrating Bcl2 rearrangement in both original and subsequently transformed neoplasm. There should be demonstration of clonal relationship by use of the same IG gene, implying a common progenitor cell. Most authors require at least 6-month interval between diagnosis of follicular lymphoma and the transformed lymphoma, though this may not always hold true as concurrent presentation of FL and transformed lymphoma has been reported [145–150].

Pathobiology of Transformation

FL is an indolent, incurable, and fatal malignancy. It arises from germinal center B cells, and the natural history includes multiple relapses and transformation. The disease has a relatively long median survival of 8–10 years, and patients have initially a high rate of response; the illness follows a fluctuating course of progression punctuated by remissions of variable duration. For as many as half of patients, transformation to a more aggressive lymphoma may occur, usually diffuse large B-cell lymphoma, and this is often associated with a particularly poor response to treatment. Transformation in FL was first reported almost 65 years ago, yet the underlying biology along with the ability to predict transformation is not well defined.

Some of the biologic factors associated with histologic transformation include secondary changes such as gains in Chr 6p, 7, 8, 12q, 18, X, and 18q and loss in 1p, 6q, 10q, and 17p [151]. Other genetic lesions in the tumor cells seen are p53 loss, p16/CDKNA loss, MYC deregulation, Bcl2/Bcl6 mutation, Del/UPD1 (TNFRSF14), UPD 16p, Del 6q, tri 7, +12/12q13-14 gain, FAS mutation, CD9 loss, and aUPD [6p, 9p, 12q,17p]. Changes occur in the non-neoplastic cells residing in the microenvironment such as increased intrafollicular CD4+ T cells, changed in the pattern of regulatory T cells, and decreased PD-1+ T-cells. These abnormalities likely impair immune surveillance, activate NF-kappa B pathway, and deregulate p53 and B-cell transcription factors [151, 152].

Indolent lymphomas pursue different pathways for transformation to an aggressive lymphoma such as Direct Evolution vs Divergent Evolution. In "Direct Evolution," FL acquires additional cytogenomic aberrancies and transforms to an aggressive lymphoma. In "Divergent Evolution," both the indolent and the transformed lymphoma originate from a separate ancestor B cell.

Different technologies have been used to assess this. Comparative genomic hybridization (CGH) analysis of sequential FL and transformed FL is used to evaluate critical copy number changes in the lymphomas. Some lymphomas show large regions of homozygosity in the absence of copy number change. Such regions, which have been termed acquired uniparental disomy (aUPD) or isodisomy, appear to be due to mitotic recombination between the two chromosomal homologues and are undetectable by CGH array technology. These genetic events can result in the selection of daughter cells made homozygous for a pre-existing mutation. Fitzgibbon et al. used single-nucleotide polymorphism (SNP) array technology to investigate regions of loss of heterozygosity in the absence of copy number change in order to establish the contribution of these abnormalities to the evolution of FL and transformed FL [152]. They found evidence for both direct and divergent evolution. A patient in their series exhibited a block of homozygosity on chr 12q in both FL and transformed FL samples, along with acquisition of several additional abnormalities within the transformed FL (chromosomes 6, 7, 8, 9, and 12) to support the concept of "Direct Evolution." In another example a patient showed a more complex pattern with the detection of abnormalities within the FL that were absent at transformation supporting the concept of lymphomas that originate from a separate ancestor B cell (Fig. 1.11).

Studies on the variable region of the IGH immunoglobulin heavy gene by Carlotte et al. [153] also hypothesized similar concepts of "Divergent" vs "Direct Evolution," albeit using different technologies. In divergent evolution a pre-B cell or

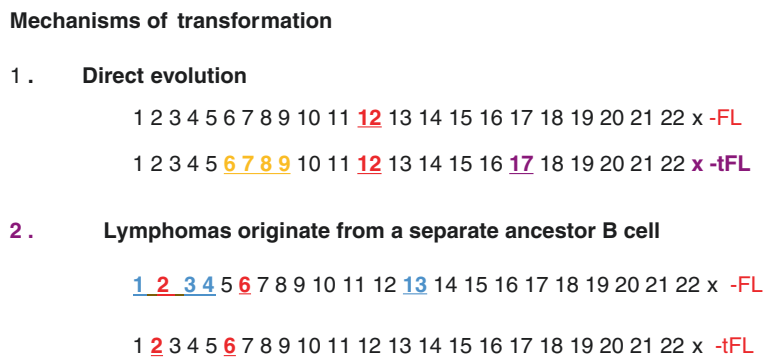

Fig. 1.11 First case: abnormalities in Chr 12 in FL and additional abnormalities in Chr 6,7,8,9,12, and 17 in tFL suggestive of Direct Evolution. Second case: numerous abnormalities in FL (Chr 1,2,3,4,6,13) and not in tFL. Detection of regions of homozygosity on 2p and 6p common to both FL and tFL suggests that these lymphomas evolved separately from an ancestor B cell. J Fitzgibbon et al. genome-wide detection of recurring sites of uniparental disomy in follicular and transformed follicular lymphoma-leukemia 2007

a common progenitor cell (CPC) acquire t(14;18) while in the bone marrow stage of development. Some of these CPCs in the germinal center reaction acquire additional clonal abnormalities to transform to follicular lymphoma, and some CPC find a niche in the bone marrow and later give rise to FL or transformed lymphoma through different pathways. In this niche, they find the ability to escape death due to treatment. Elegant integrated genomic studies by Okosun J et al. [154] identified recurrent mutations and evolution patterns in FL. Whole-genome sequencing ([154] WGS) and whole-exome sequencing (WES) studies were performed to detect non-synonymous mutations in paired FL and transformed FL(tFL). Phylogenetic trees constructed with the detected somatic variants revealed branching rather than linear evolution, with the "trunk" representing genetic events shared by the FL tumor(s) and its paired tFL, thus supporting the presence of an ancestral CPC clone. A significant pattern that emerged showed that the CPC that was rich in mutated genes demonstrated high clonal resemblance in FL and tFL and harbored mutations that were enriched for genes involved in chromatin regulation (*MLL2* and other histone-modifying genes *CREBBP*, *EZH2*), immune modulation (*TNFRSF14*), JAK-STAT, and BCR/NF-κB signaling (*BCL10*, *CARD11*, *CD79B*). These observations also showed that the lymphomas are dependent on *CREBBP*, *MLL2*, and *TNFRSF14* alterations during lymphomagenesis and progression [154]. Variant allele frequencies (VAFs) are used to discriminate early from late genetic events as mutations present in nearly all tumor cells (clonal) suggest early events and therefore represent initiating "driver" genes. Their results showed that the histone-modifying genes (*MLL2*, *CREBBP*, and *EZH2*), *STAT6*, and *TNFRSF14* were "driver" genes and a subset of these (*CREBBP*, *MLL2*, *TNFRSF14*) harbored compound heterozygous mutations that were frequently either accompanied by deletions or acquired uniparental disomy (aUPD) and render tumor cells homozygous for a pre-existing abnormality. Further, mutations in these genes were stable and remained clonally dominant irrespective of therapy. Novel transformation-associated events include copy number alterations (CNAs) with amplifications of EZH2, *MDM2*, *MYC*, and *REL. These* "progressor" abnormalities were not seen in the antecedent FL biopsies and therefore appear to have been gained during the most recent clonal expansion prior to transformation. In summary, FL undergoes genetic evolution over time and favors a path of divergent evolution rather than direct evolution. Multiple subclones may develop over time. Transformation may develop in an earlier common progenitor rather than a later subclone [155, 156] (Figs. 1.12 and 1.13).

Case-Continued

The patient subsequently presented with large inguinal mass (Fig. 1.14).

The lymph node architecture was effaced by a diffuse population of medium- to large-sized monomorphous cells with fine chromatin, small single nucleoli, and variable amount of cytoplasm. The cells exhibited squared off borders of retracted cytoplasm. Numerous mitoses, prominent apoptosis, and background sclerosis were also seen. The tumor cells were diffusely positive for CD20, CD10, BCL2, and

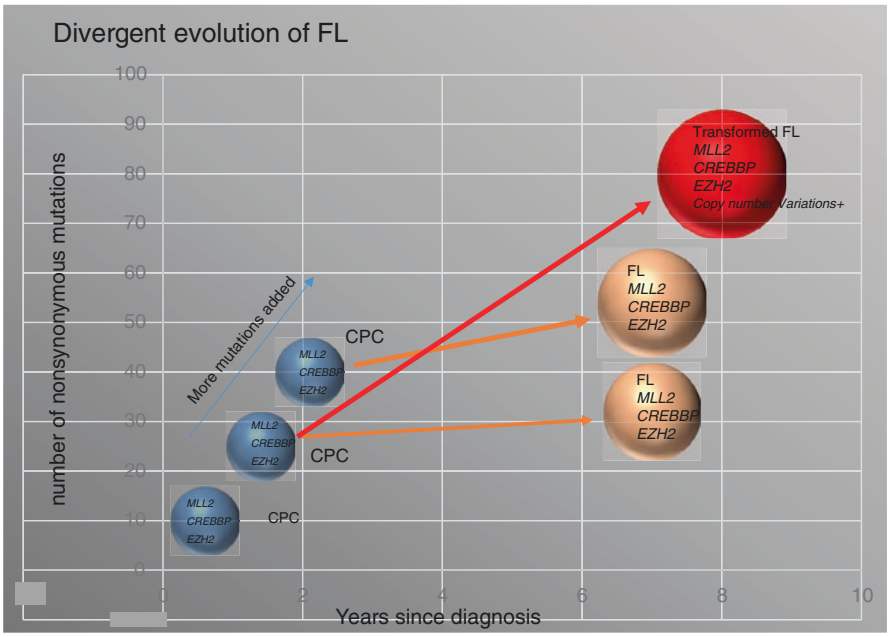

Fig. 1.12 Divergent Evolution pattern of FL. In "Divergent Evolution," both the indolent and the transformed lymphoma originate from a separate ancestor B cell. (Adapted from Okosun et al. [154])

- Genetic lesions in the tumor cells
  - P53 loss, p16/CDKNA loss, MYC deregulation, Bcl2/Bcl6 mutation, Del/UPD1 (TNFRSF14), UPD 16p, Del 6q, tri 7, +12/12q13-14 gain, FAS mutation, CD9 loss, aUPD[6p, 9p, 12q,17p]
- Changes in microenvironment (non-neoplastic cells)
  - FDC loss or immaturity, Increased intrafollicular CD4+ T cells, pattern of regulatory T cells, Decreased PD-1+ T-cells, Increased MVD
- Abnormalities likely impair immune surveillance, activate NF-kappa B pathway, deregulate p53 and B-cell transcription factors

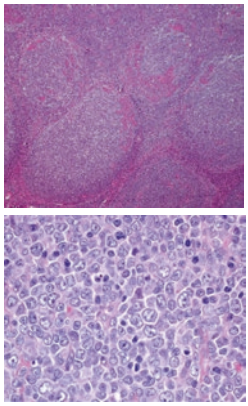

Fig. 1.13 Biologic factors associated with histologic transformation. (Lossos and Goscoyne [150], Bouska [151], Fitzgibbon et al. [152])

BCL6 with a Ki-67 proliferative index of 95–100%. CD3 highlighted reactive small T lymphocytes. The neoplastic lymphocytes were negative for TDT, CyclinD1, and MUM1. Interphase fluorescence in situ hybridization (FISH) performed on the inguinal lymph node with commercial MYC Dual Color Break Apart Rearrangement Probe and BCL2 Dual Color Break Apart Rearrangement Probe revealed a signal pattern consistent with rearrangement of one MYC locus and rearrangement of one

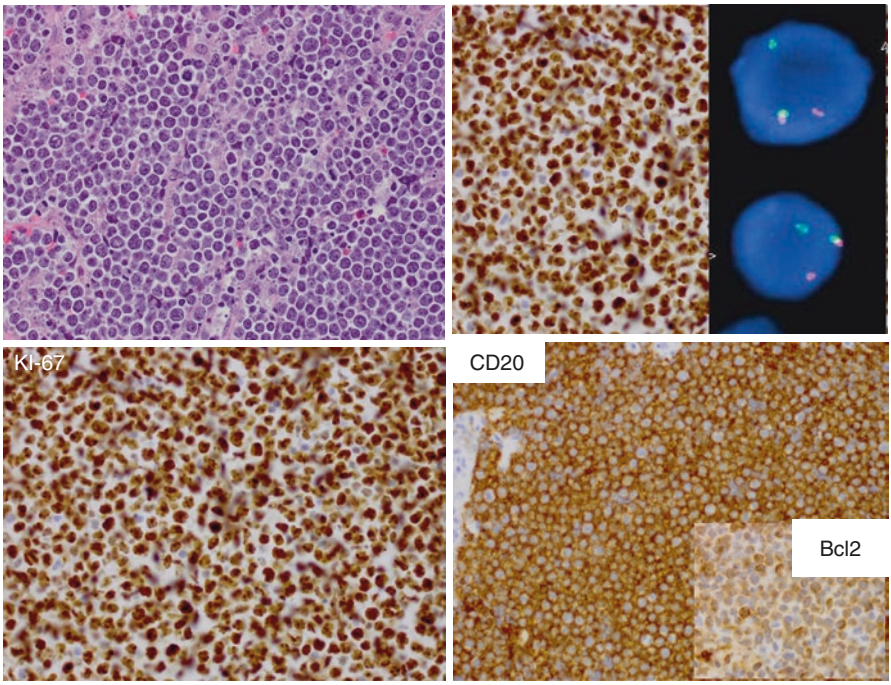

Fig. 1.14 Transformation to high-grade B-cell lymphoma, MYC+, Bcl2+

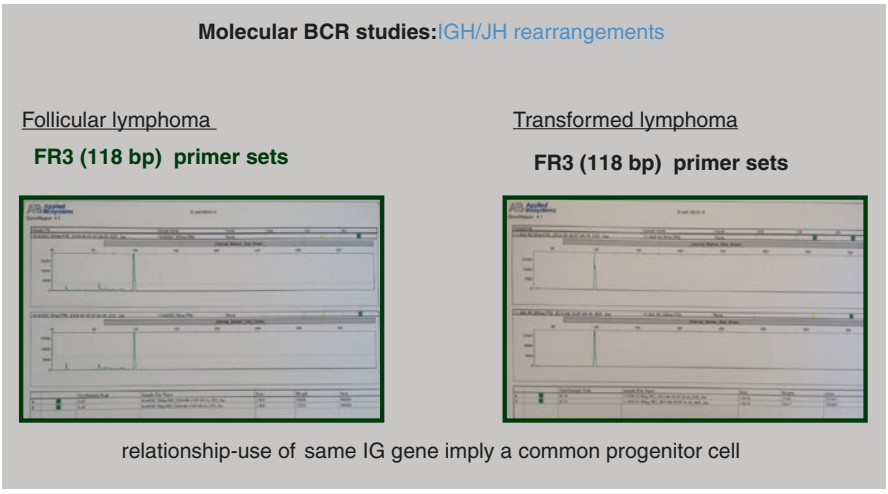

Fig. 1.15 Use of same IG gene implies a common progenitor cell for the follicular lymphoma and transformed high-grade B-cell lymphoma

BCL2 locus (Fig. 1.14). Molecular PCR studies showed the same B-cell receptor clone in FL and tFL, supporting a clonal relationship. The diagnosis was compatible with *high-grade B-cell lymphoma* (Figs. 1.14 and 1.15).

Other Entities

Pediatric-Type Follicular Lymphoma (PTFL)

PTFL shows genetic profile that is quite distinct from FL and lacks the usual translocations found in other B-cell lymphomas of germinal center origin such as Bcl2 and BCl6 rearrangements. The t(14;18) is almost completely absent in FL below the age of 18. In contrast to adult cases, pediatric FLs are also more frequently grade 3 or composite FL/DLBCL and have a significantly better 5-year event-free survival. Therefore, "pediatric FL" has been considered as distinct variant of FL by the updated World Health Organization classification [157, 158].

PTFLs occur primarily in children and young adults. It is reported, rarely, in older individuals. PTFLs involve lymph nodes of the head and neck region (cervical, submental, submandibular, postauricular, and periparotid nodes) with inguinal region rarely involved. It is usually a stage 1 disease. Systemic lymph node involvement (radiological evidence of mediastinal disease or intra-abdominal involvement) or extra nodal disease, or areas of DLBCL, precludes this diagnosis. The median age is 15–18 years and mostly <40 years. The male to female ratio is 10:1. The lymph node architecture is effaced by large ill-defined follicles with attenuated lymphoid cuffs. The follicles are often serpiginous or irregularly shaped. A prominent starry sky pattern is present. Occasional nodal PFL cases can show more uniform, closely packed follicles. A peripheral rim of paler cells suggesting marginal zone differentiation is sometimes present but is usually a minor component. The cytological composition is dominated by monotonous, small- to medium-sized blastoid cells with round to oval nuclei, finely dispersed chromatin, small nucleoli, and scant cytoplasm. Typical centroblasts with peripheral membrane-bound nuclei are infrequent. Centrocyte-like cells can be seen but with somewhat dispersed chromatin. This blastoid appearance correlates with a high proliferative rate. Cytologically these have been reported as high grade (grade 3A, 3B), and grading is not typically done [159].

The phenotype is CD20+/CD79a+/ PAX5+/ Cd10+/ Bcl6+, and Bcl2 is negative or weak positive. Ki-67 is usually >30% without polarization within the follicles. FDC marker CD21, CD23, highlight FDC meshworks within the follicles. IRF4/MUM is negative. Plasma cells are sparse. Rare cases of florid follicular hyperplasia can demonstrate CD10+ monotypic B cells as analyzed by flow cytometry. Hence architectural effacement is key to the diagnosis [160]. These cases demonstrate clonality by PCR techniques and are useful in ruling out reactive follicular hyperplasia [160].

Genetics These lymphomas do not show aberrancies in Bcl2, Bcl6, IRF4, KMT2D (MLL2), CREBBP, and EZH2 that are commonly seen in FLs [161]. The most common genetic aberration is del 1p36 or mutations affecting TNGRSF14 and mutations in MAP 2K1 [162]. Prognosis is excellent, and treatment may be limited to excision alone without definite need for radiation or chemotherapy.

Large B-Cell Lymphoma with IRF4/MUM1 Rearrangement

LBCL with IRF4 is uncommon and seen in less than 1% of DLBCLs. It presents primarily in children and young adults. The median age is 12 years, although the age range is wide from 4 to 79 years. It is usually a low-stage disease (stage 1–2), and the sites involved are Waldeyer's ring or head and neck lymph nodes. It can be entirely diffuse, diffuse and follicular, or entirely follicular. Of note, as LBCL-IRF4/MUM1+ can present with a "follicular only" morphology, it raises the differential of follicular lymphoma. It is for this reason that LBCL-IRF4+ is discussed here.

The cells are medium to large size with more open chromatin than seen in centrocytes and with small basophilic nucleoli. Mitotic figures are not significant, and starry sky pattern is not seen. When this presents in a follicular pattern, it is usually back-to-back with attenuated mantles. The serpiginous pattern is not common. The immunophenotype is CD20+/MUM1+/CD10+/ Bcl6+/ Bcl2(+/−). Proliferation fraction is high. The immunoglobulin genes are clonally rearranged. A cytogenetically cryptic rearrangement of IRF4 with IgH locus is seen (sometimes light chains are involved) and is delineated by FISH studies. Bcl6 rearrangements can be seen. There is absence of PRDM1/BLIMP1. MYC and Bcl2 rearrangements are not seen. These cases have an IHC and gene expression signature that is distinct from both germinal center B cells and activated B cells The LBCL-IRF4 can be placed in the GC subtype by the gold-standard gene expression profiling studies yet lack CD10 protein expression. These lymphomas show strong MUM1 positivity and therefore assigned to the non-GCB subtype according to the immunohistochemistry-based Hans algorithm yielding conflicting results between both classifiers. They also express CD5, a marker that identifies a distinct subgroup of DLBCL. Both features, the lack in correlation of gene expression and immunohistochemical classification and the high rate of CD5 positivity in CD10-negative lymphomas, perhaps indicate that *IG/IRF4*-positive lymphomas constitute a separate subgroup of mature B-cell lymphomas. The follicular morphology can be similar with the PTFL (CD20+/CD10+/Bcl6+/Bcl2-neg/IRF4/MUM1 neg); however the cytology of medium to large cells, lack of serpiginous growth pattern, helps to differentiate from pediatric-type FL. There is difference in clinical behavior between the two entities and hence the need to differentiate from PTFL. The LBCL-IR4 has good prognosis *after* combination immunochemotherapy with or without radiation, while pediatric-type FL has good prognosis with local management. Therefore it is prudent to look for IRF4 rearrangements by FISH in lesions that present in the head and neck, Waldeyer's region: should the morphology and clinical presentation be compatible with LBCL-IRF4/MUM1+ type.

Summarizing all pediatric lesions such as pediatric-type FL, LBCL-IR4, and testicular FL, they usually present as low-stage, high-grade disease and tend to do well with a more favorable outcome as compared to adults, be it with chemo or local treatment alone. This may be due to differences in host characteristics, tumor biology, including the presence of chromosomal alterations, suggesting that diversity in tumor biology might contribute to the age-dependent differences in prognosis of lymphoma [159, 163].

Prognosis and Risk Stratification

Prognosis is closely linked to the extent of disease at presentation. FLIPI, a modification of International Prognostic Index (IPI), is useful in predicting outcome. Follicular Lymphoma International Prognostic Index prognostic model for FL uses five independent predictors of inferior survival: age (>60 years), Ann Arbor stage (III–IV), hemoglobin level (<12 g/dL), number of nodal areas ≥5), and serum LDH level (above normal). Three risk groups are defined: low risk (0–1 adverse factor), intermediate risk (2 factors), and poor risk (> or = 3 adverse factors) [164]. Additional prognostic indices have modified FLIPI, adding cardiovascular disease [165]. Later a scoring system was designed for de novo follicular lymphoma treated initially with immunotherapy and included β2-microglobulin and bone marrow involvement in PRIMA-PI (posttreatment prognostic indicator named after PRIMA trial) [165]. Molecular models (m7-FLIPI) use mutational status based on next-generation sequencing of seven genes [102]. This includes the mutation status of seven genes (EZH2, ARID1A, MEF2B, EP300, FOXO1, CREBBP, and CARD11), the Follicular Lymphoma International Prognostic Index (FLIPI), and Eastern Cooperative Oncology Group (ECOG) performance status (Fig. 1.16).

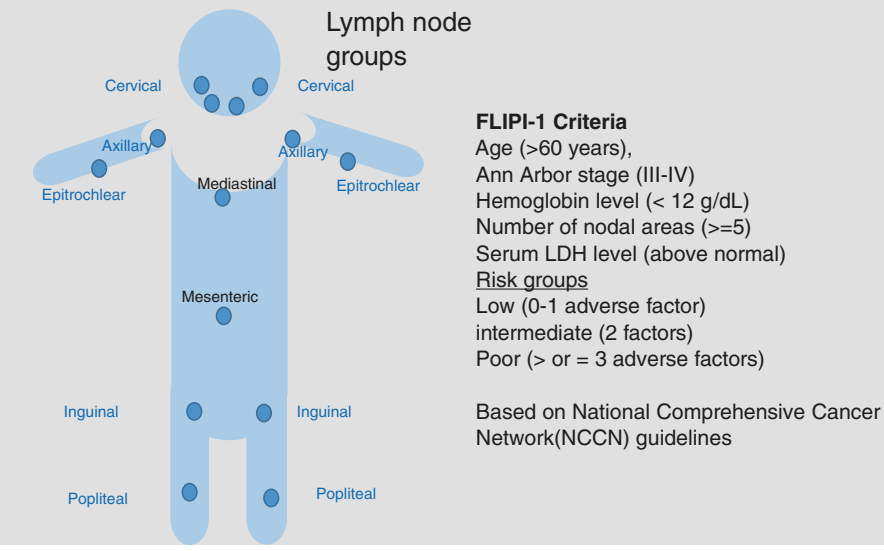

Fig. 1.16 NCCN guidelines for staging and the use of FLIPI criteria for prognostication

Summary

- FL is a lymphoma of GC origin, CD10+, and Bcl6+, with >85% demonstrating chromosomal translocation t(14;18)(q32;q21), involving *BCL2* and *IGH genes. The lymphoma shows two cell types – centrocytes and centroblasts. Grading is based on number of centroblasts/HPF averaged over 10HPF (grade 1–2 < 15/HPF; grade 3 with > 15/HPF)*: *grade 3A lymphomas contain centrocytes while grade 3B lymphomas do not show intervening centrocytes.*
- Three patterns are recognized: follicular, follicular and diffuse, and diffuse.
- Partial involvement by FL is considered a form of FL for clinical purposes and should not be confused with in situ follicular neoplasia.
- *Four variants are recognized*:
  - "In situ follicular neoplasia (formerly called "follicular lymphoma in situ"; these are tissue-based precursor lesions and biologically similar to blood-based Bcl2 rearrangement carrying cells referred to as FL-like B cells.
  - Duodenal type FL: localized, good prognosis.
  - Testicular FL: children, high grade, good prognosis.
  - Diffuse variant of FL.
- *IP and morphologically distinct subtypes of FL*:
  - FL with marginal zone differentiation demonstrate neoplastic cells with marginal zone morphology and also show lack of CD10 within a FL case that carries classic CD20+/CD10+ "FL cells"
  - CD10-/MUM1 positive FL.
  - Floral variant, CD5+.
  - Diffuse follicular lymphoma (CD20+/CD10+/ CD23+); negative for t(14;18) with IgH:Bcl2 rearrangement; presents primarily in the inguinal region; similar to FL by gene expression profiling studies.
- *Molecular distinct FL entities – reason for classifying them separately from FL*:
  - Pediatric-type FLs – lack the gene expression profile seen in FL.
  - Primary cutaneous FLs – lack the gene expression profile seen in FL and instead show a gene expression profile similar to DLBCL-GC type with amplification of *REL* gene.

    Of note, CD10-negative/MUM1-positive/Bcl2-negative phenotype FL is classified as FL because of its genetic similarity to classic FL.
- *Pediatric entities* – these are usually low-stage, high-grade, Bcl2-negative entities, good prognosis:
  - Pediatric-type FL (PTFL): CD10+/ (IRF4/MUM1) negative, lymph nodes, treatment can be limited to local resection.
  - Testicular FL, treatment with local resection.
  - Purely follicular LBCL-IRF4+: CD10-/(IRF/MUM1)+, Waldeyer's ring and lymph nodes, treatment with immunochemo/radiation.

- *CD10-/MUM1*-positive lymphomas include two separate entities:
  - *CD10-/MUM1*+ FL, high grade, elderly, poor survival
  - *CD10-/MUM1*+ LBCL, subset present with purely follicular pattern, young adults, good prognosis
- *Normal B-cell development*. B lymphocytes mature in the bone marrow and in the process rearrange immunoglobulin variable (V) gene segments to create an immunoglobulin molecule that serves as the B-cell receptor for antigen. When an antigen of adequate affinity engages the receptor, the cell enters a germinal center in lymphoid follicles, where its V genes undergo somatic hypermutation. This process introduces mutations in the rearranged VHDJH and VLJL gene segments. Cells with receptors that have enhanced antigen-binding affinity proliferate in the presence of the antigen, whereas cells with receptors that no longer bind the antigen or do bind autoantigens are eliminated. The germinal center pathways require the help of T lymphocytes. The process can proceed without T cells and outside germinal centers, in the marginal zones around lymphoid follicles, most often in response to carbohydrates of encapsulated bacteria or viruses. Both processes lead to the development of plasma cells or memory (antigen-experienced) B cells [27].
- *Follicular lymphomagenesis and transformation.* The t(14;18) translocation occurs during VDJ recombination at the pre-B-cell stage, leading to constitutive expression of the anti-apoptotic protein BCL2. Pre-B cells then migrate to the GC of the lymph node as a naive B cell. Bcl2 expression rescues t(14;18)-bearing cells, which allow them to exit the GC reaction as switched but immunoglobulin M-expressing (sIgM) (through the "allelic paradox") memory B cells. In the GC genomic remodeling and intense proliferation take place, and some of these t(14;18)-bearing cells might give rise to follicular lymphoma (FL)-like cells (FLLCs) characterized by a centroblast-like or centrocyte-like phenotype and constitutive activation-induced cytidine deaminase (AID) and B-cell lymphoma 6 protein (BCL-6) activity. At each developmental step, FLLCs might disseminate extensively and traffic between follicles, blood, and bone marrow compartments. Reentry in the germinal center induces clonal expansion and acquisition of additional genomic alterations. FL-like cells (FLLCs) are present in most healthy individuals and do not progress to FL. At some point these become committed precursor cells (CPCs) that ultimately give rise to FL. With additional genetic mutations, they may develop into in situ follicular neoplasia (ISFN) cells, or FL with partial involvement or overt FL. In FL, spatial and temporal genomic analysis reveals that relapse events or transformations to high- grade FL arise predominantly by divergent evolution of a common mutated CPC that clonally diverges through the acquisition of distinct genetic events. Relapse events arising from a direct clonal evolution of the dominant FL clones present at the diagnosis also exist. For direct evolution the CPC is postulated to reside in the normal GC, in FL, and tFL. Or the premalignant CPC resides in the normal GC or BM and can independently give rise to FL or tFL [10, 81, 153].

References

1. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375–90.
2. Ottensmeier CH, Thompsett AR, Zhu D, Wilkins BS, Sweetenham JW, Stevenson FK. Analysis of VH genes in follicular and diffuse lymphoma shows ongoing somatic mutation and multiple isotype transcripts in early disease with changes during disease progression. Blood. 1998;91(11):4292–9.
3. Cleary ML, Meeker TC, Levy S, Lee E, Trela M, Sklar J, Levy R. Clustering of extensive somatic mutations in the variable region of an immunoglobulin heavy chain gene from a human B cell lymphoma. Cell. 1986;44(1):97–106.
4. Teras LR, DeSantis CE, Cerhan JR, Morton LM, Jemal A, Flowers CR. 2016 US lymphoid malignancy statistics by World Health Organization subtypes. CA Cancer J Clin. 2016;66(6):443–59.
5. Clarke CA, Glaser SL, Gomez SL, Wang SS, Keegan TH, Yang J, Chang ET. Lymphoid malignancies in U.S. Asians: incidence rate differences by birthplace and acculturation. Cancer Epidemiol Biomarkers Prev. 2011;20(6):1064–77.
6. Agrawal R, Wang J. Pediatric follicular lymphoma: a rare clinicopathologic entity. Arch Pathol Lab Med. 2009;133(1):142–6.
7. Linet MS, Vajdic CM, Morton LM, de Roos AJ, Skibola CF, Boffetta P, Cerhan JR, Flowers CR, de Sanjose S, Monnereau A, et al. Medical history, lifestyle, family history, and occupational risk factors for follicular lymphoma: the InterLymph Non-Hodgkin Lymphoma Subtypes Project. J Natl Cancer Inst Monogr. 2014;2014(48):26–40.
8. Wang SS, Carrington M, Berndt SI, Slager SL, Bracci PM, Voutsinas J, Cerhan JR, Smedby KE, Hjalgrim H, Vijai J, et al. HLA class I and II diversity contributes to the etiologic heterogeneity of non-Hodgkin lymphoma subtypes. Cancer Res. 2018;78(14):4086–96.
9. Skibola CF, Slager SL, Berndt SI, Lightfoot T, Sampson JN, Morton LM, Weisenburger DD. Medical history, lifestyle, family history, and occupational risk factors for adult acute lymphocytic leukemia: the InterLymph Non-Hodgkin Lymphoma Subtypes Project. J Natl Cancer Inst Monogr. 2014;2014(48):125–9.
10. Carbone A, Roulland S, Gloghini A, Younes A, von Keudell G, Lopez-Guillermo A, Fitzgibbon J. Follicular lymphoma. Nat Rev Dis Primers. 2019;5(1):83.
11. Agopian J, Navarro JM, Gac AC, Lecluse Y, Briand M, Grenot P, Gauduchon P, Ruminy P, Lebailly P, Nadel B, et al. Agricultural pesticide exposure and the molecular connection to lymphomagenesis. J Exp Med. 2009;206(7):1473–83.
12. Chiu BC, Dave BJ, Blair A, Gapstur SM, Zahm SH, Weisenburger DD. Agricultural pesticide use and risk of t(14;18)-defined subtypes of non-Hodgkin lymphoma. Blood. 2006;108(4):1363–9.
13. Basten A, Brink R, Peake P, Adams E, Crosbie J, Hartley S, Goodnow CC. Self tolerance in the B-cell repertoire. Immunol Rev. 1991;122:5–19.
14. Nemazee D, Russell D, Arnold B, Haemmerling G, Allison J, Miller JF, Morahan G, Buerki K. Clonal deletion of autospecific B lymphocytes. Immunol Rev. 1991;122:117–32.
15. Bachmann MF, Rohrer UH, Kundig TM, Burki K, Hengartner H, Zinkernagel RM. The influence of antigen organization on B cell responsiveness. Science. 1993;262(5138):1448–51.
16. Reth M, Hombach J, Wienands J, Campbell KS, Chien N, Justement LB, Cambier JC. The B-cell antigen receptor complex. Immunol Today. 1991;12(6):196–201.
17. Brack C, Hirama M, Lenhard-Schuller R, Tonegawa S. A complete immunoglobulin gene is created by somatic recombination. Cell. 1978;15(1):1–14.
18. Camacho FI, Algara P, Rodriguez A, Ruiz-Ballesteros E, Mollejo M, Martinez N, Martinez-Climent JA, Gonzalez M, Mateo M, Caleo A, et al. Molecular heterogeneity in MCL defined by the use of specific VH genes and the frequency of somatic mutations. Blood. 2003;101(10):4042–6.

19. Alt FW, Yancopoulos GD, Blackwell TK, Wood C, Thomas E, Boss M, Coffman R, Rosenberg N, Tonegawa S, Baltimore D. Ordered rearrangement of immunoglobulin heavy chain variable region segments. EMBO J. 1984;3(6):1209–19.
20. Kelsoe G. B cell diversification and differentiation in the periphery. J Exp Med. 1994;180(1):5–6.
21. MacLennan IC. Germinal centers. Annu Rev Immunol. 1994;12:117–39.
22. de Vinuesa CG, Cook MC, Ball J, Drew M, Sunners Y, Cascalho M, Wabl M, Klaus GG, MacLennan IC. Germinal centers without T cells. J Exp Med. 2000;191(3):485–94.
23. Monson NL, Foster SJ, Brezinschek HP, Brezinschek RI, Dorner T, Lipsky PE. The role of CD40-CD40 ligand (CD154) interactions in immunoglobulin light chain repertoire generation and somatic mutation. Clin Immunol. 2001;100(1):71–81.
24. Weller S, Faili A, Garcia C, Braun MC, Le Deist FF, de Saint Basile GG, Hermine O, Fischer A, Reynaud CA, Weill JC. CD40-CD40L independent Ig gene hypermutation suggests a second B cell diversification pathway in humans. Proc Natl Acad Sci U S A. 2001;98(3):1166–70.
25. Toellner KM, Jenkinson WE, Taylor DR, Khan M, Sze DM, Sansom DM, Vinuesa CG, MacLennan IC. Low-level hypermutation in T cell-independent germinal centers compared with high mutation rates associated with T cell-dependent germinal centers. J Exp Med. 2002;195(3):383–9.
26. William J, Euler C, Christensen S, Shlomchik MJ. Evolution of autoantibody responses via somatic hypermutation outside of germinal centers. Science. 2002;297(5589):2066–70.
27. Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. N Engl J Med. 2005;352(8):804–15.
28. Rajewsky K. Clonal selection and learning in the antibody system. Nature. 1996;381(6585):751–8.
29. Meffre E, Casellas R, Nussenzweig MC. Antibody regulation of B cell development. Nat Immunol. 2000;1(5):379–85.
30. Maul RW, Gearhart PJ. AID and somatic hypermutation. Adv Immunol. 2010;105:159–91.
31. Teng G, Papavasiliou FN. Immunoglobulin somatic hypermutation. Annu Rev Genet. 2007;41:107–20.
32. Di Noia JM, Neuberger MS. Molecular mechanisms of antibody somatic hypermutation. Annu Rev Biochem. 2007;76:1–22.
33. Zeng X, Winter DB, Kasmer C, Kraemer KH, Lehmann AR, Gearhart PJ. DNA polymerase eta is an A-T mutator in somatic hypermutation of immunoglobulin variable genes. Nat Immunol. 2001;2(6):537–41.
34. Bebenek K, Tissier A, Frank EG, McDonald JP, Prasad R, Wilson SH, Woodgate R, Kunkel TA. 5′-Deoxyribose phosphate lyase activity of human DNA polymerase iota in vitro. Science. 2001;291(5511):2156–9.
35. Stavnezer J, Guikema JE, Schrader CE. Mechanism and regulation of class switch recombination. Annu Rev Immunol. 2008;26:261–92.
36. Methot SP, Di Noia JM. Molecular mechanisms of somatic hypermutation and class switch recombination. Adv Immunol. 2017;133:37–87.
37. Steele EJ, Franklin A, Blanden RV. Genesis of the strand-biased signature in somatic hypermutation of rearranged immunoglobulin variable genes. Immunol Cell Biol. 2004;82(2):209–18.
38. Mayorov VI, Rogozin IB, Adkison LR, Gearhart PJ. DNA polymerase eta contributes to strand bias of mutations of A versus T in immunoglobulin genes. J Immunol. 2005;174(12):7781–6.
39. Tippin B, Pham P, Goodman MF. Error-prone replication for better or worse. Trends Microbiol. 2004;12(6):288–95.
40. Pavlov YI, Rogozin IB, Galkin AP, Aksenova AY, Hanaoka F, Rada C, Kunkel TA. Correlation of somatic hypermutation specificity and A-T base pair substitution errors by DNA polymerase eta during copying of a mouse immunoglobulin kappa light chain transgene. Proc Natl Acad Sci U S A. 2002;99(15):9954–9.
41. Chang B, Casali P. The CDR1 sequences of a major proportion of human germline Ig VH genes are inherently susceptible to amino acid replacement. Immunol Today. 1994;15(8):367–73.

42. Jukes TH, King JL. Evolutionary nucleotide replacements in DNA. Nature. 1979;281(5732):605–6.
43. Nagasawa T. Microenvironmental niches in the bone marrow required for B-cell development. Nat Rev Immunol. 2006;6(2):107–16.
44. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity. 2006;25(6):977–88.
45. Mackarehtschian K, Hardin JD, Moore KA, Boast S, Goff SP, Lemischka IR. Targeted disruption of the flk2/flt3 gene leads to deficiencies in primitive hematopoietic progenitors. Immunity. 1995;3(1):147–61.
46. Opferman JT, Iwasaki H, Ong CC, Suh H, Mizuno S, Akashi K, Korsmeyer SJ. Obligate role of anti-apoptotic MCL-1 in the survival of hematopoietic stem cells. Science. 2005;307(5712):1101–4.
47. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci U S A. 1998;95(7):3597–602.
48. Koopman G, Parmentier HK, Schuurman HJ, Newman W, Meijer CJ, Pals ST. Adhesion of human B cells to follicular dendritic cells involves both the lymphocyte function-associated antigen 1/intercellular adhesion molecule 1 and very late antigen 4/vascular cell adhesion molecule 1 pathways. J Exp Med. 1991;173(6):1297–304.
49. Koopman G, Keehnen RM, Lindhout E, Newman W, Shimizu Y, van Seventer GA, de Groot C, Pals ST. Adhesion through the LFA-1 (CD11a/CD18)-ICAM-1 (CD54) and the VLA-4 (CD49d)-VCAM-1 (CD106) pathways prevents apoptosis of germinal center B cells. J Immunol. 1994;152(8):3760–7.
50. Wykes M, Poudrier J, Lindstedt R, Gray D. Regulation of cytoplasmic, surface and soluble forms of CD40 ligand in mouse B cells. Eur J Immunol. 1998;28(2):548–59.
51. Wykes M. Why do B cells produce CD40 ligand? Immunol Cell Biol. 2003;81(4):328–31.
52. Armitage RJ, Fanslow WC, Strockbine L, Sato TA, Clifford KN, Macduff BM, Anderson DM, Gimpel SD, Davis-Smith T, Maliszewski CR, et al. Molecular and biological characterization of a murine ligand for CD40. Nature. 1992;357(6373):80–2.
53. Fuleihan R, Ramesh N, Geha RS. Role of CD40-CD40-ligand interaction in Ig-isotype switching. Curr Opin Immunol. 1993;5(6):963–7.
54. Grabstein KH, Maliszewski CR, Shanebeck K, Sato TA, Spriggs MK, Fanslow WC, Armitage RJ. The regulation of T cell-dependent antibody formation in vitro by CD40 ligand and IL-2. J Immunol. 1993;150(8 Pt 1):3141–7.
55. Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev. 2009;229(1):152–72.
56. Cyster JG, Ansel KM, Reif K, Ekland EH, Hyman PL, Tang HL, Luther SA, Ngo VN. Follicular stromal cells and lymphocyte homing to follicles. Immunol Rev. 2000;176:181–93.
57. Harwood NE, Batista FD. New insights into the early molecular events underlying B cell activation. Immunity. 2008;28(5):609–19.
58. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. Blood. 2008;112(5):1570–80.
59. Gatto D, Brink R. The germinal center reaction. J Allergy Clin Immunol. 2010;126(5):898–907; quiz 908-899.
60. MacLennan IC. Germinal centers still hold secrets. Immunity. 2005;22(6):656–7.
61. Schmidlin H, Diehl SA, Blom B. New insights into the regulation of human B-cell differentiation. Trends Immunol. 2009;30(6):277–85.
62. Gold MR, Matsuuchi L, Kelly RB, DeFranco AL. Tyrosine phosphorylation of components of the B-cell antigen receptors following receptor crosslinking. Proc Natl Acad Sci U S A. 1991;88(8):3436–40.

63. Rolli V, Gallwitz M, Wossning T, Flemming A, Schamel WW, Zurn C, Reth M. Amplification of B cell antigen receptor signaling by a Syk/ITAM positive feedback loop. Mol Cell. 2002;10(5):1057–69.
64. Fruman DA, Satterthwaite AB, Witte ON. Xid-like phenotypes: a B cell signalosome takes shape. Immunity. 2000;13(1):1–3.
65. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell. 1997;91(2):231–41.
66. Weng WK, Jarvis L, LeBien TW. Signaling through CD19 activates Vav/mitogen-activated protein kinase pathway and induces formation of a CD19/Vav/phosphatidylinositol 3-kinase complex in human B cell precursors. J Biol Chem. 1994;269(51):32514–21.
67. Hashimoto A, Okada H, Jiang A, Kurosaki M, Greenberg S, Clark EA, Kurosaki T. Involvement of guanosine triphosphatases and phospholipase C-gamma2 in extracellular signal-regulated kinase, c-Jun NH2-terminal kinase, and p38 mitogen-activated protein kinase activation by the B cell antigen receptor. J Exp Med. 1998;188(7):1287–95.
68. Barkett M, Gilmore TD. Control of apoptosis by Rel/NF-kappaB transcription factors. Oncogene. 1999;18(49):6910–24.
69. Aarts WM, Bende RJ, Steenbergen EJ, Kluin PM, Ooms EC, Pals ST, van Noesel CJ. Variable heavy chain gene analysis of follicular lymphomas: correlation between heavy chain isotype expression and somatic mutation load. Blood. 2000;95(9):2922–9.
70. Smit LA, Bende RJ, Aten J, Guikema JE, Aarts WM, van Noesel CJ. Expression of activation-induced cytidine deaminase is confined to B-cell non-Hodgkin's lymphomas of germinal-center phenotype. Cancer Res. 2003;63(14):3894–8.
71. Cleary ML, Smith SD, Sklar J. Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t(14;18) translocation. Cell. 1986;47(1):19–28.
72. Bakhshi A, Wright JJ, Graninger W, Seto M, Owens J, Cossman J, Jensen JP, Goldman P, Korsmeyer SJ. Mechanism of the t(14;18) chromosomal translocation: structural analysis of both derivative 14 and 18 reciprocal partners. Proc Natl Acad Sci U S A. 1987;84(8):2396–400.
73. Weinberg OK, Ai WZ, Mariappan MR, Shum C, Levy R, Arber DA. "Minor" BCL2 breakpoints in follicular lymphoma: frequency and correlation with grade and disease presentation in 236 cases. J Mol Diagn. 2007;9(4):530–7.
74. Cleary ML, Galili N, Sklar J. Detection of a second t(14;18) breakpoint cluster region in human follicular lymphomas. J Exp Med. 1986;164(1):315–20.
75. Albinger-Hegyi A, Hochreutener B, Abdou MT, Hegyi I, Dours-Zimmermann MT, Kurrer MO, Heitz PU, Zimmermann DR. High frequency of t(14;18)-translocation breakpoints outside of major breakpoint and minor cluster regions in follicular lymphomas: improved polymerase chain reaction protocols for their detection. Am J Pathol. 2002;160(3):823–32.
76. Ngan BY, Nourse J, Cleary ML. Detection of chromosomal translocation t(14;18) within the minor cluster region of bcl-2 by polymerase chain reaction and direct genomic sequencing of the enzymatically amplified DNA in follicular lymphomas. Blood. 1989;73(7):1759–62.
77. McDonnell TJ, Deane N, Platt FM, Nunez G, Jaeger U, McKearn JP, Korsmeyer SJ. bcl-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. Cell. 1989;57(1):79–88.
78. Limpens J, Stad R, Vos C, de Vlaam C, de Jong D, van Ommen GJ, Schuuring E, Kluin PM. Lymphoma-associated translocation t(14;18) in blood B cells of normal individuals. Blood. 1995;85(9):2528–36.
79. Roulland S, Lebailly P, Roussel G, Briand M, Cappellen D, Pottier D, Hardouin A, Troussard X, Bastard C, Henry-Amar M, et al. BCL-2/JH translocation in peripheral blood lymphocytes of unexposed individuals: lack of seasonal variations in frequency and molecular features. Int J Cancer. 2003;104(6):695–8.
80. Roulland S, Navarro JM, Grenot P, Milili M, Agopian J, Montpellier B, Gauduchon P, Lebailly P, Schiff C, Nadel B. Follicular lymphoma-like B cells in healthy individuals: a novel intermediate step in early lymphomagenesis. J Exp Med. 2006;203(11):2425–31.

81. Huet S, Sujobert P, Salles G. From genetics to the clinic: a translational perspective on follicular lymphoma. Nat Rev Cancer. 2018;18(4):224–39.
82. Kishimoto W, Nishikori M. Molecular pathogenesis of follicular lymphoma. J Clin Exp Hematop. 2014;54(1):23–30.
83. Vaandrager JW, Schuuring E, Kluin-Nelemans HC, Dyer MJ, Raap AK, Kluin PM. DNA fiber fluorescence in situ hybridization analysis of immunoglobulin class switching in B-cell neoplasia: aberrant CH gene rearrangements in follicle center-cell lymphoma. Blood. 1998;92(8):2871–8.
84. Akasaka T, Akasaka H, Yonetani N, Ohno H, Yamabe H, Fukuhara S, Okuma M. Refinement of the BCL2/immunoglobulin heavy chain fusion gene in t(14;18)(q32;q21) by polymerase chain reaction amplification for long targets. Genes Chromosomes Cancer. 1998;21(1):17–29.
85. Sungalee S, Mamessier E, Morgado E, Gregoire E, Brohawn PZ, Morehouse CA, Jouve N, Monvoisin C, Menard C, Debroas G, et al. Germinal center reentries of BCL2-overexpressing B cells drive follicular lymphoma progression. J Clin Invest. 2014;124(12):5337–51.
86. Ramiro AR, Jankovic M, Eisenreich T, Difilippantonio S, Chen-Kiang S, Muramatsu M, Honjo T, Nussenzweig A, Nussenzweig MC. AID is required for c-myc/IgH chromosome translocations in vivo. Cell. 2004;118(4):431–8.
87. Mlynarczyk C, Fontan L, Melnick A. Germinal center-derived lymphomas: the darkest side of humoral immunity. Immunol Rev. 2019;288(1):214–39.
88. Pandey S, Mourcin F, Marchand T, Nayar S, Guirriec M, Pangault C, Monvoisin C, Ame-Thomas P, Guilloton F, Dulong J, et al. IL-4/CXCL12 loop is a key regulator of lymphoid stroma function in follicular lymphoma. Blood. 2017;129(18):2507–18.
89. Ame-Thomas P, Tarte K. The yin and the yang of follicular lymphoma cell niches: role of microenvironment heterogeneity and plasticity. Semin Cancer Biol. 2014;24:23–32.
90. Dogan I, Bertocci B, Vilmont V, Delbos F, Megret J, Storck S, Reynaud CA, Weill JC. Multiple layers of B cell memory with different effector functions. Nat Immunol. 2009;10(12):1292–9.
91. Amin R, Mourcin F, Uhel F, Pangault C, Ruminy P, Dupre L, Guirriec M, Marchand T, Fest T, Lamy T, et al. DC-SIGN-expressing macrophages trigger activation of mannosylated IgM B-cell receptor in follicular lymphoma. Blood. 2015;126(16):1911–20.
92. Linley A, Krysov S, Ponzoni M, Johnson PW, Packham G, Stevenson FK. Lectin binding to surface Ig variable regions provides a universal persistent activating signal for follicular lymphoma cells. Blood. 2015;126(16):1902–10.
93. McCann KJ, Ottensmeier CH, Callard A, Radcliffe CM, Harvey DJ, Dwek RA, Rudd PM, Sutton BJ, Hobby P, Stevenson FK. Remarkable selective glycosylation of the immunoglobulin variable region in follicular lymphoma. Mol Immunol. 2008;45(6):1567–72.
94. Coelho V, Krysov S, Ghaemmaghami AM, Emara M, Potter KN, Johnson P, Packham G, Martinez-Pomares L, Stevenson FK. Glycosylation of surface Ig creates a functional bridge between human follicular lymphoma and microenvironmental lectins. Proc Natl Acad Sci U S A. 2010;107(43):18587–92.
95. Boice M, Salloum D, Mourcin F, Sanghvi V, Amin R, Oricchio E, Jiang M, Mottok A, Denis-Lagache N, Ciriello G, et al. Loss of the HVEM tumor suppressor in lymphoma and restoration by modified CAR-T cells. Cell. 2016;167(2):405–418.e413.
96. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, Paul JE, Boyle M, Woolcock BW, Kuchenbauer F, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet. 2010;42(2):181–5.
97. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, Johnson NA, Severson TM, Chiu R, Field M, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature. 2011;476(7360):298–303.
98. Ennishi D, Takata K, Beguelin W, Duns G, Mottok A, Farinha P, Bashashati A, Saberi S, Boyle M, Meissner B, et al. Molecular and genetic characterization of MHC deficiency identifies EZH2 as therapeutic target for enhancing immune recognition. Cancer Discov. 2019;9(4):546–63.

99. Mondello P, Tadros S, Teater M, Fontan L, Chang AY, Jain N, Yang H, Singh S, Ying HY, Chu CS, et al. Selective inhibition of HDAC3 targets synthetic vulnerabilities and activates immune surveillance in lymphoma. Cancer Discov. 2020;10(3):440–59.
100. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, Kasper LH, Lerach S, Tang H, Ma J, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. Nature. 2011;471(7337):189–95.
101. Karube K, Enjuanes A, Dlouhy I, Jares P, Martin-Garcia D, Nadeu F, Ordonez GR, Rovira J, Clot G, Royo C, et al. Integrating genomic alterations in diffuse large B-cell lymphoma identifies new relevant pathways and potential therapeutic targets. Leukemia. 2018;32(3):675–84.
102. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger AM, Szczepanowski M, Pott C, Kopp N, Murakami M, Horn H, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol. 2015;16(9):1111–22.
103. Krysiak K, Gomez F, White BS, Matlock M, Miller CA, Trani L, Fronick CC, Fulton RS, Kreisel F, Cashen AF, et al. Recurrent somatic mutations affecting B-cell receptor signaling pathway genes in follicular lymphoma. Blood. 2017;129(4):473–83.
104. Okosun J, Wolfson RL, Wang J, Araf S, Wilkins L, Castellano BM, Escudero-Ibarz L, Al Seraihi AF, Richter J, Bernhart SH, et al. Recurrent mTORC1-activating RRAGC mutations in follicular lymphoma. Nat Genet. 2016;48(2):183–8.
105. Hoglund M, Sehn L, Connors JM, Gascoyne RD, Siebert R, Sall T, Mitelman F, Horsman DE. Identification of cytogenetic subgroups and karyotypic pathways of clonal evolution in follicular lymphomas. Genes Chromosomes Cancer. 2004;39(3):195–204.
106. Mann RB, Berard CW. Criteria for the cytologic subclassification of follicular lymphomas: a proposed alternative method. Hematol Oncol. 1983;1(2):187–92.
107. Weigert O, Kopp N, Lane AA, Yoda A, Dahlberg SE, Neuberg D, Bahar AY, Chapuy B, Kutok JL, Longtine JA, et al. Molecular ontogeny of donor-derived follicular lymphomas occurring after hematopoietic cell transplantation. Cancer Discov. 2012;2(1):47–55.
108. Dreyling M, Ghielmini M, Rule S, Salles G, Vitolo U, Ladetto M. Newly diagnosed and relapsed follicular lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017;28(12):3109.
109. Wang SA, Wang L, Hochberg EP, Muzikansky A, Harris NL, Hasserjian RP. Low histologic grade follicular lymphoma with high proliferation index: morphologic and clinical features. Am J Surg Pathol. 2005;29(11):1490–6.
110. Dogan A, Du MQ, Aiello A, Diss TC, Ye HT, Pan LX, Isaacson PG. Follicular lymphomas contain a clonally linked but phenotypically distinct neoplastic B-cell population in the interfollicular zone. Blood. 1998;91(12):4708–14.
111. Gradowski JF, Jaffe ES, Warnke RA, Pittaluga S, Surti U, Gole LA, Swerdlow SH. Follicular lymphomas with plasmacytic differentiation include two subtypes. Mod Pathol. 2010;23(1):71–9.
112. Bosga-Bouwer AG, van den Berg A, Haralambieva E, de Jong D, Boonstra R, Kluin P, van den Berg E, Poppema S. Molecular, cytogenetic, and immunophenotypic characterization of follicular lymphoma grade 3B; a separate entity or part of the spectrum of diffuse large B-cell lymphoma or follicular lymphoma? Hum Pathol. 2006;37(5):528–33.
113. Menter T, Gasser A, Juskevicius D, Dirnhofer S, Tzankov A. Diagnostic utility of the germinal center-associated markers GCET1, HGAL, and LMO2 in hematolymphoid neoplasms. Appl Immunohistochem Mol Morphol. 2015;23(7):491–8.
114. Li Y, Hu S, Zuo Z, Hong M, Lin P, Li S, Konoplev S, Wang Z, Khoury JD, Young KH, et al. CD5-positive follicular lymphoma: clinicopathologic correlations and outcome in 88 cases. Mod Pathol. 2015;28(6):787–98.
115. Tiesinga JJ, Wu CD, Inghirami G. CD5+ follicle center lymphoma. Immunophenotyping detects a unique subset of "floral" follicular lymphoma. Am J Clin Pathol. 2000;114(6):912–21.

116. Karube K, Guo Y, Suzumiya J, Sugita Y, Nomura Y, Yamamoto K, Shimizu K, Yoshida S, Komatani H, Takeshita M, et al. CD10-MUM1+ follicular lymphoma lacks BCL2 gene translocation and shows characteristic biologic and clinical features. Blood. 2007;109(7):3076–9.
117. Kendrick SL, Redd L, Muranyi A, Henricksen LA, Stanislaw S, Smith LM, Perry AM, Fu K, Weisenburger DD, Rosenwald A, et al. BCL2 antibodies targeted at different epitopes detect varying levels of protein expression and correlate with frequent gene amplification in diffuse large B-cell lymphoma. Hum Pathol. 2014;45(10):2144–53.
118. van Dongen JJ, Langerak AW, Bruggemann M, Evans PA, Hummel M, Lavender FL, Delabesse E, Davi F, Schuuring E, Garcia-Sanz R, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia. 2003;17(12):2257–317.
119. Payne K, Wright P, Grant JW, Huang Y, Hamoudi R, Bacon CM, Du MQ, Liu H. BIOMED-2 PCR assays for IGK gene rearrangements are essential for B-cell clonality analysis in follicular lymphoma. Br J Haematol. 2011;155(1):84–92.
120. Einerson RR, Kurtin PJ, Dayharsh GA, Kimlinger TK, Remstein ED. FISH is superior to PCR in detecting t(14;18)(q32;q21)-IgH/bcl-2 in follicular lymphoma using paraffin-embedded tissue samples. Am J Clin Pathol. 2005;124(3):421–9.
121. Nathwani BN, Metter GE, Miller TP, Burke JS, Mann RB, Barcos M, Kjeldsberg CR, Dixon DO, Winberg CD, Whitcomb CC, et al. What should be the morphologic criteria for the subdivision of follicular lymphomas? Blood. 1986;68(4):837–45.
122. Salaverria I, Siebert R. Follicular lymphoma grade 3B. Best Pract Res Clin Haematol. 2011;24(2):111–9.
123. Wahlin BE, Yri OE, Kimby E, Holte H, Delabie J, Smeland EB, Sundstrom C, Christensson B, Sander B. Clinical significance of the WHO grades of follicular lymphoma in a population-based cohort of 505 patients with long follow-up times. Br J Haematol. 2012;156(2):225–33.
124. Shustik J, Quinn M, Connors JM, Gascoyne RD, Skinnider B, Sehn LH. Follicular non-Hodgkin lymphoma grades 3A and 3B have a similar outcome and appear incurable with anthracycline-based therapy. Ann Oncol. 2011;22(5):1164–9.
125. Adam P, Katzenberger T, Eifert M, Ott MM, Rosenwald A, Muller-Hermelink HK, Ott G. Presence of preserved reactive germinal centers in follicular lymphoma is a strong histopathologic indicator of limited disease stage. Am J Surg Pathol. 2005;29(12):1661–4.
126. Katzenberger T, Kalla J, Leich E, Stocklein H, Hartmann E, Barnickel S, Wessendorf S, Ott MM, Muller-Hermelink HK, Rosenwald A, et al. A distinctive subtype of t(14;18)-negative nodal follicular non-Hodgkin lymphoma characterized by a predominantly diffuse growth pattern and deletions in the chromosomal region 1p36. Blood. 2009;113(5):1053–61.
127. Wang E, West D, Kulbacki E. An unusual nodal marginal zone lymphoma with bright CD10 expression: a potential diagnostic pitfall. Am J Hematol. 2010;85(7):546–8.
128. Goodlad JR, Batstone PJ, Hamilton D, Hollowood K. Follicular lymphoma with marginal zone differentiation: cytogenetic findings in support of a high-risk variant of follicular lymphoma. Histopathology. 2003;42(3):292–8.
129. Nathwani BN, Anderson JR, Armitage JO, Cavalli F, Diebold J, Drachenberg MR, Harris NL, MacLennan KA, Muller-Hermelink HK, Ullrich FA, et al. Clinical significance of follicular lymphoma with monocytoid B cells. Non-Hodgkin's lymphoma classification project. Hum Pathol. 1999;30(3):263–8.
130. Dogan A, Bagdi E, Munson P, Isaacson PG. CD10 and BCL-6 expression in paraffin sections of normal lymphoid tissue and B-cell lymphomas. Am J Surg Pathol. 2000;24(6):846–52.
131. Marafioti T, Copie-Bergman C, Calaminici M, Paterson JC, Shende VH, Liu H, Baia M, Ramsay AD, Agostinelli C, Briere J, et al. Another look at follicular lymphoma: immunophenotypic and molecular analyses identify distinct follicular lymphoma subgroups. Histopathology. 2013;62(6):860–75.
132. Pileri SA, Sabattini E, Rosito P, Zinzani PL, Ascani S, Fraternali-Orcioni G, Gamberi B, Piccioli M, Vivenza D, Falini B, et al. Primary follicular lymphoma of the testis in childhood: an entity with peculiar clinical and molecular characteristics. J Clin Pathol. 2002;55(9):684–8.

133. Finn LS, Viswanatha DS, Belasco JB, Snyder H, Huebner D, Sorbara L, Raffeld M, Jaffe ES, Salhany KE. Primary follicular lymphoma of the testis in childhood. Cancer. 1999;85(7):1626–35.
134. Lones MA, Raphael M, McCarthy K, Wotherspoon A, Terrier-Lacombe MJ, Ramsay AD, Maclennan K, Cairo MS, Gerrard M, Michon J, et al. Primary follicular lymphoma of the testis in children and adolescents. J Pediatr Hematol Oncol. 2012;34(1):68–71.
135. Tsuboi K, Iida S, Inagaki H, Kato M, Hayami Y, Hanamura I, Miura K, Harada S, Kikuchi M, Komatsu H, et al. MUM1/IRF4 expression as a frequent event in mature lymphoid malignancies. Leukemia. 2000;14(3):449–56.
136. Falini B, Fizzotti M, Pucciarini A, Bigerna B, Marafioti T, Gambacorta M, Pacini R, Alunni C, Natali-Tanci L, Ugolini B, et al. A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. Blood. 2000;95(6):2084–92.
137. Karube K, Ying G, Tagawa H, Niino D, Aoki R, Kimura Y, Hashikawa K, Suefuji N, Sugita Y, Nomura Y, et al. BCL6 gene amplification/3q27 gain is associated with unique clinicopathological characteristics among follicular lymphoma without BCL2 gene translocation. Mod Pathol. 2008;21(8):973–8.
138. Bermudez G, Gonzalez de Villambrosia S, Martinez-Lopez A, Batlle A, Revert-Arce JB, Cereceda Company L, Ortega Bezanilla C, Piris MA, Montes-Moreno S. Incidental and isolated follicular lymphoma in situ and mantle cell lymphoma in situ lack clinical significance. Am J Surg Pathol. 2016;40(7):943–9.
139. Mamessier E, Broussais-Guillaumot F, Chetaille B, Bouabdallah R, Xerri L, Jaffe ES, Nadel B. Nature and importance of follicular lymphoma precursors. Haematologica. 2014;99(5):802–10.
140. Cheung MC, Bailey D, Pennell N, Imrie KR, Berinstein NL, Amato D, Ghorab Z. In situ localization of follicular lymphoma: evidence for subclinical systemic disease with detection of an identical BCL-2/IGH fusion gene in blood and lymph node. Leukemia. 2009;23(6):1176–9.
141. Takata K, Okada H, Ohmiya N, Nakamura S, Kitadai Y, Tari A, Akamatsu T, Kawai H, Tanaka S, Araki H, et al. Primary gastrointestinal follicular lymphoma involving the duodenal second portion is a distinct entity: a multicenter, retrospective analysis in Japan. Cancer Sci. 2011;102(8):1532–6.
142. Takata K, Sato Y, Nakamura N, Tokunaka M, Miki Y, Yukie Kikuti Y, Igarashi K, Ito E, Harigae H, Kato S, et al. Duodenal follicular lymphoma lacks AID but expresses BACH2 and has memory B-cell characteristics. Mod Pathol. 2013;26(1):22–31.
143. Bende RJ, Smit LA, Bossenbroek JG, Aarts WM, Spaargaren M, de Leval L, Boeckxstaens GE, Pals ST, van Noesel CJ. Primary follicular lymphoma of the small intestine: alpha4beta7 expression and immunoglobulin configuration suggest an origin from local antigen-experienced B cells. Am J Pathol. 2003;162(1):105–13.
144. Schmatz AI, Streubel B, Kretschmer-Chott E, Puspok A, Jager U, Mannhalter C, Tiemann M, Ott G, Fischbach W, Herzog P, et al. Primary follicular lymphoma of the duodenum is a distinct mucosal/submucosal variant of follicular lymphoma: a retrospective study of 63 cases. J Clin Oncol. 2011;29(11):1445–51.
145. Brunner P, Rufle A, Dirnhofer S, Lohri A, Willi N, Cathomas G, Tzankov A, Juskevicius D. Follicular lymphoma transformation into histiocytic sarcoma: indications for a common neoplastic progenitor. Leukemia. 2014;28(9):1937–40.
146. LeBrun DP, Ngan BY, Weiss LM, Huie P, Warnke RA, Cleary ML. The bcl-2 oncogene in Hodgkin's disease arising in the setting of follicular non-Hodgkin's lymphoma. Blood. 1994;83(1):223–30.
147. Brauninger A, Hansmann ML, Strickler JG, Dummer R, Burg G, Rajewsky K, Kuppers R. Identification of common germinal-center B-cell precursors in two patients with both Hodgkin's disease and non-Hodgkin's lymphoma. N Engl J Med. 1999;340(16):1239–47.
148. Raffeld M, Wright JJ, Lipford E, Cossman J, Longo DL, Bakhshi A, Korsmeyer SJ. Clonal evolution of t(14;18) follicular lymphomas demonstrated by immunoglobulin genes and the 18q21 major breakpoint region. Cancer Res. 1987;47(10):2537–42.

149. De Jong D, Voetdijk BM, Beverstock GC, van Ommen GJ, Willemze R, Kluin PM. Activation of the c-myc oncogene in a precursor-B-cell blast crisis of follicular lymphoma, presenting as composite lymphoma. N Engl J Med. 1988;318(21):1373–8.
150. Lossos IS, Gascoyne RD. Transformation of follicular lymphoma. Best Pract Res Clin Haematol. 2011;24(2):147–63.
151. Bouska A, McKeithan TW, Deffenbacher KE, Lachel C, Wright GW, Iqbal J, Smith LM, Zhang W, Kucuk C, Rinaldi A, et al. Genome-wide copy-number analyses reveal genomic abnormalities involved in transformation of follicular lymphoma. Blood. 2014;123(11):1681–90.
152. Fitzgibbon J, Iqbal S, Davies A, O'Shea D, Carlotti E, Chaplin T, Matthews J, Raghavan M, Norton A, Lister TA, et al. Genome-wide detection of recurring sites of uniparental disomy in follicular and transformed follicular lymphoma. Leukemia. 2007;21(7):1514–20.
153. Carlotti E, Wrench D, Matthews J, Iqbal S, Davies A, Norton A, Hart J, Lai R, Montoto S, Gribben JG, et al. Transformation of follicular lymphoma to diffuse large B-cell lymphoma may occur by divergent evolution from a common progenitor cell or by direct evolution from the follicular lymphoma clone. Blood. 2009;113(15):3553–7.
154. Okosun J, Bodor C, Wang J, Araf S, Yang CY, Pan C, Boller S, Cittaro D, Bozek M, Iqbal S, et al. Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma. Nat Genet. 2014;46(2):176–81.
155. Green MR, Alizadeh AA. Common progenitor cells in mature B-cell malignancies: implications for therapy. Curr Opin Hematol. 2014;21(4):333–40.
156. Green MR, Gentles AJ, Nair RV, Irish JM, Kihira S, Liu CL, Kela I, Hopmans ES, Myklebust JH, Ji H, et al. Hierarchy in somatic mutations arising during genomic evolution and progression of follicular lymphoma. Blood. 2013;121(9):1604–11.
157. Lorsbach RB, Shay-Seymore D, Moore J, Banks PM, Hasserjian RP, Sandlund JT, Behm FG. Clinicopathologic analysis of follicular lymphoma occurring in children. Blood. 2002;99(6):1959–64.
158. Oschlies I, Salaverria I, Mahn F, Meinhardt A, Zimmermann M, Woessmann W, Burkhardt B, Gesk S, Krams M, Reiter A, et al. Pediatric follicular lymphoma--a clinico-pathological study of a population-based series of patients treated within the non-Hodgkin's lymphoma--Berlin-Frankfurt-Munster (NHL-BFM) multicenter trials. Haematologica. 2010;95(2):253–9.
159. Liu Q, Salaverria I, Pittaluga S, Jegalian AG, Xi L, Siebert R, Raffeld M, Hewitt SM, Jaffe ES. Follicular lymphomas in children and young adults: a comparison of the pediatric variant with usual follicular lymphoma. Am J Surg Pathol. 2013;37(3):333–43.
160. Kussick SJ, Kalnoski M, Braziel RM, Wood BL. Prominent clonal B-cell populations identified by flow cytometry in histologically reactive lymphoid proliferations. Am J Clin Pathol. 2004;121(4):464–72.
161. Green MR, Kihira S, Liu CL, Nair RV, Salari R, Gentles AJ, Irish J, Stehr H, Vicente-Duenas C, Romero-Camarero I, et al. Mutations in early follicular lymphoma progenitors are associated with suppressed antigen presentation. Proc Natl Acad Sci U S A. 2015;112(10):E1116–25.
162. Louissaint A Jr, Ackerman AM, Dias-Santagata D, Ferry JA, Hochberg EP, Huang MS, Iafrate AJ, Lara DO, Pinkus GS, Salaverria I, et al. Pediatric-type nodal follicular lymphoma: an indolent clonal proliferation in children and adults with high proliferation index and no BCL2 rearrangement. Blood. 2012;120(12):2395–404.
163. Salaverria I, Philipp C, Oschlies I, Kohler CW, Kreuz M, Szczepanowski M, Burkhardt B, Trautmann H, Gesk S, Andrusiewicz M, et al. Translocations activating IRF4 identify a subtype of germinal center-derived B-cell lymphoma affecting predominantly children and young adults. Blood. 2011;118(1):139–47.
164. Solal-Celigny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, Au WY, Bellei M, Brice P, Caballero D, et al. Follicular lymphoma international prognostic index. Blood. 2004;104(5):1258–65.
165. van de Schans SA, Steyerberg EW, Nijziel MR, Creemers GJ, Janssen-Heijnen ML, van Spronsen DJ. Validation, revision and extension of the follicular lymphoma international prognostic index (FLIPI) in a population-based setting. Ann Oncol. 2009;20(10):1697–702.

Chapter 2
Mantle Cell Lymphoma: Definition, Epidemiology, Pathobiology – Lymphomagenesis, Morphology, Variants, Immunophenotype, Prognostic Factors

Olga V. Danilova

Case Presentation

The 68-year-old male presented to the primary care provider with unintentional weight loss, fevers and sweats at night, and malaise. Physical exam revealed palpable cervical, axillary, and inguinal lymph nodes. Complete blood cell count was performed and has the following results: white blood cell count 10.6×10^3/microliter, hemoglobin 9.2 g/dL, and platelets 98×10^3/microliter. Positron emission tomography-computed tomography[1] showed extensive and bulky cervical, axillary, mediastinal, abdominal, pelvic, and inguinal lymphadenopathy with standardized uptake value up to 14–17.

The biopsy of the axillary lymph node showed a diffuse proliferation of small- to medium-sized lymphocytes with slightly irregular nuclear profiles. The neoplastic lymphocytes were positive for CD20, CD5, cyclin D1, and BCL2 and showed a low Ki-67 proliferation index. Fluorescent in situ hybridization demonstrated IGH-CCND1 fusion. The findings support the diagnosis of mantle cell lymphoma (Fig. 2.1).

[1] PET scan uses radiotracers, mostly commonly similar to glucose F-18 fluorodeoxyglucose, or FDG, linked to a small amount of radioactive material that can be detected on the PET scan. Metabolically active lymphoma cells absorb glucose at higher rate than normal cells, hence making lymphoma cells "hot" on PET scan with much higher FDG levels compared to normal cells.

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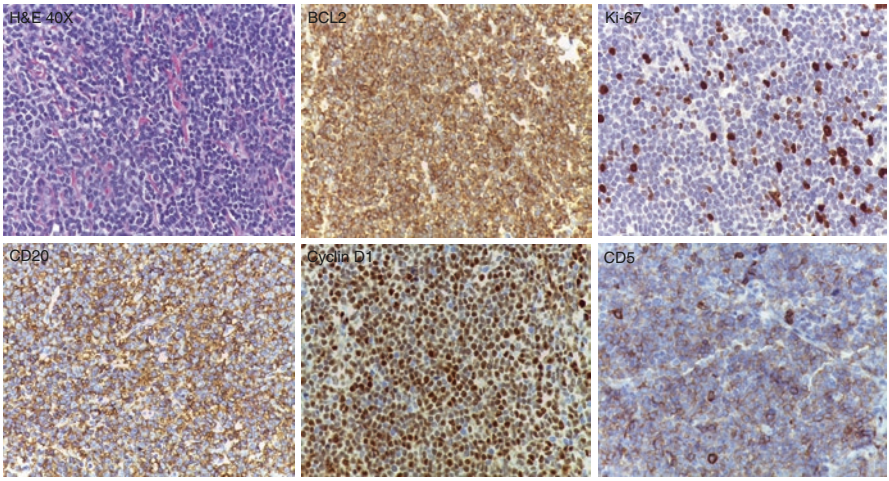

Fig. 2.1 Case presentation. Monotonous proliferation of small- to medium-sized lymphoid cells. Immunophenotypes CD20, CD5, cyclin D1, Bcl2, low Ki-67 proliferation index

Definition and Historical Overview

Current 2016 WHO classification [1, 2] defines mantle cell lymphoma (MCL) as mature B-cell neoplasm usually composed of monomorphic small- to medium-sized lymphoid cells with irregular nuclear contours. Neoplastic transformed cells (centroblasts), paraimmunoblasts, and proliferation centers are absent. MCL was first described almost three decades ago as the centrocytic lymphoma (CCL) and was initially recognized morphologically. It was described by Lennert as a "diffuse germinocytoma" in 1973 [3]. In a 1975 publication, diffuse germinocytoma was described as a tumor that "looks very much like CLL" but lacks proliferation centers [4]. The cells were described as lymphocytes slightly larger than CLL cells with nuclei that are "very irregular in shape (not round) and often cleaved" with "very fine" chromatin and very small to absent nucleoli and scant slightly basophilic cytoplasm. The centrocytic lymphoma(CCL) was noted to differ from cleaved cell lymphomas (now known as follicular lymphomas) in lacking "germinoblasts" (centroblasts/transformed follicular center cells) and was considered a critical feature in distinguishing CCL from other cleaved cell lymphomas. The morphologic delineation of centrocytes versus centroblastic-centrocytic lymphomas (CBCCL) became the basis of Kiel classification. The term *centrocytic lymphoma* was first used in the 1974 European Lymphoma Club publication of the Kiel classification [3]. Later, based on similarity of CCL cells to centrocytes of reactive germinal centers (cleaved nuclei, presence of Slg and both complement-receptor subtypes, and absence of receptors for mouse E), it was concluded by Lennert that this lymphoma is closely related or derived from centrocytes of germinal centers. Again, diffuse architectural effacement was noted, and complete absence of centroblast-like and

follicle mantle lymphocyte-like cells was highlighted. Per Lennert's observation, the neoplasia was beginning within and around germinal centers with wide mantle of centrocytes around residual germinal centers [5].

Another distinguishing difference was while CBCCL was noted to transform to centroblastic (large noncleaved lymphomas), such transformation was almost never observed in CCL. More pronounced nuclear polymorphism and more numerous mitoses were described in CCL compared to CBCCLCCL which could be distinguished from B-CLL based on differences in complement receptor expression, a lack of mouse erythrocyte rosettes, and its stronger surface immunoglobulin expression [5, 6]. Stansfeld et al. in 1983 noted variations in cell size, nuclear irregularity, and nuclear dispersal. Overall, its distinct clinical behavior as more aggressive than B-CLL and CBCCL was noted. It was further noted that lymphomas with small round cell morphology showed an indolent course, while the more aggressive cases demonstrated high mitotic rate and blast-appearing nuclei [6]. With the introduction of immunohistochemistry in the 1980s, the B-cell origin was confirmed, and the cells were immunophenotyped as CD5 positive in contrast to the CD10-positive cells of follicular center origin. However, the presence of CD5-positive B cells was suggested in the germinal follicles, and, therefore, it was concluded that while CCL cells are related to the follicle, they could be easily placed into either the mantle or germinal center compartment especially considering some overlapping morphologic characteristics. Nevertheless, there was clear immunophenotypic evidence to separate CCL from CBCCL. Harris et al. in 1984 confirmed the immunophenotypic features of CCL and described pattern of CCL as "large or small irregular aggregates" of follicular dendritic cells, as opposed to the discrete staining in the nodules of follicular lymphomas. Distinct clinical features were also described as this lymphoma was seen in older individuals, with a marked male predominance, who presented with adenopathy, often hepatosplenomegaly, including sometimes massively enlarged spleens, and frequent bone marrow involvement and had shorter survival [7].

Swerdlow et al. in 1993 performed a morphometric study of CCL and other small-cleaved follicular center lymphomas. This study showed that on average, the CCLs were composed of cells that had larger, less irregular, less oval, and more monomorphic nuclei. No difference was noted in the proportion of admixed small round lymphocytes [8, 9]. Banks et al. postulated that the small cells of CCL may be the neoplastic counterpart of cells normally residing in the follicle mantle. The term "mantle cell" lymphoma was introduced to replace CCL. The study recognized Lennert's morphologic description of mantle cell lymphoma as one of the best with addition that infrequently residual nonneoplastic germinal centers may be present. This change in terminology clearly highlighted the now well-accepted fact that the so-called centrocytes in CCL were very different from those in CBCCL and that the neoplastic cells in CCL were more closely related to mantle cells than to FCC. The term *centrocytic* was therefore no longer appropriate [9].

In 1992, the International Lymphoma Study Group proposed that the term *mantle cell lymphoma* replaces centrocytic lymphoma term along with other terminology used for this entity (mantle zone lymphoma, malignant lymphoma, intermediate

differentiation) based on understanding that the cells in this disease are a neoplastic counterpart of cells normally residing in the follicle mantle. Identification of cyclin D1 and its pathogenic role further defined mantle cell lymphoma as a distinct clinicopathologic entity [9].

Morphologic and immunophenotypic features were further defined at a workshop organized by the European Task Force on Lymphoma, Annecy, France, in 1994 [10]. It was stated that morphologically typical MCL could have either a nodular or a diffuse growth pattern, with or without residual germinal centers, and with either loose or tighter follicular dendritic cell (FDC) aggregates. Only nodular cases with residual germinal centers were considered to have a mantle zone growth pattern. Cytologically, the neoplastic cells were described as small- to medium-sized with irregular nuclei (or mixture of irregular and round), fine condensed chromatin, small inconspicuous nucleoli, and pale indistinct/scant cytoplasm. Centroblasts-immunoblasts were rare to absent. Typically, the neoplastic cells were CD5, CD20, and bcl-2 positive monoclonal B cells with strong surface IgM and IgD but were negative for CD23, CD10, CD11c, and CD25. Loosely organized FDC meshworks were usually present. Already discovered at that time, cyclin D1 overexpression and the t(11;14)(q13;q32) were considered characteristic of MCL, but their documentation was not considered a requirement for the diagnosis, and their specificity was not established. Variations in the cytology included cases with predominantly round cells and cases with predominantly or admixed blastoid cells. Whether there was a variant with more abundant pale cytoplasm was considered uncertain. Phenotypic variations included more variable CD5, CD23, and possibly CD10 expression. At that time, diagnosis of MCL was believed to require either typical histopathology or a typical phenotype. It was noted that genotypic or cytogenetic studies might be of use in identifying variant or atypical cases in the future.

The median survival was reported to be approximately 3–4 years. No specific treatment guidelines could be recommended. From a pathologist's perspective, the European Lymphoma Task Force workshop was important in recognizing that MCL showed greater morphologic heterogeneity than had originally been described. It also highlighted unanswered questions, some of which remain unresolved at the present time [10].

Based on its morphology, MCL was historically discussed together with the indolent forms of NHL, but the behavior of classic MCL is more often that of an aggressive lymphoma. Recently more indolent variants such as *leukemic non-nodal and in situ mantle cell neoplasia are* described.

Epidemiology and Clinical Features

Currently MCL accounts for approximately 3–10% of adult NHLs in the United States and Europe [2, 11]. MCL predominantly occurs in elderly men (male to female ratio ≥2:1, male predominance remains unexplained) with a median age at diagnosis of 65–70 years. The mean annual incidence of this lymphoma has been

estimated at 0.42 per 100,000 (range, 0.38–0.49), with a mean of 0.7 for men and 0.2 for women. Patients with mantle cell lymphoma may have relatives with other lymphomas, highlighting family predisposition for lymphoid neoplasms. Clinically, MCL frequently presents in advanced stage with generalized lymphadenopathy, hepatosplenomegaly, and bone marrow involvement. Hepatosplenomegaly occurs relatively commonly (30–60%), and splenic rupture may be the initial presentation of the disease. Extranodal and peripheral blood involvement is not unusual especially in cases with extensive lymphadenopathy [2, 3, 6, 12–16].

While extranodal involvement is frequent in MCL, extranodal presentation without apparent nodal involvement occurs in only 4–15% of cases. Gastrointestinal involvement has been reported in 10–25% of patients. Lymphomatoid polyposis is a distinctive gastrointestinal presentation with multiple lymphoid polyps found in the small and large bowel. Central nervous system involvement occurs in 10–20% of patients and is usually seen later in the clinical course of resistant disease or generalized relapse, advanced-stage disease with extensive infiltration in other extranodal localizations, and a leukemic phase [17–20].

Other extranodal sites commonly involved are Waldeyer's ring, lung, and pleura (5–20%). The skin, breast, soft tissue, thyroid, salivary gland, peripheral nerve, conjunctiva, and orbit involvement is much less common [12, 16, 21].

Typical laboratory findings are anemia and thrombocytopenia (10–40% of patients), high lactate dehydrogenase[2], and β_2-microglobulin levels (an indication of high turnover of lymphoma cells) (~50% of cases). A monoclonal paraprotein can be detected in serum of 10–30% of patients but usually at low levels, and the immunoglobulin isotype in the serum and on the cell surface may be different. MCL patients have increased incidence of second malignancies in 12–21% of patients, with a predilection for urinary tract [12, 13, 16, 21–23].

Atypical lymphoid cells may be observed in the peripheral blood in the absence of lymphocytosis, and they may be detected by flow cytometry in virtually all patients.

As mentioned above, leukemic involvement may present in the later stages of disease progression or may represent a manifestation of disease progression. Some patients present with a very aggressive leukemic form mimicking acute leukemia. These cases have blastoid morphology; complex karyotypes, occasionally with 8q24 anomalies; and a very rapid evolution, with a median survival of only 3 months.

A very distinct group of patients are the ones who present with leukemic involvement and no or minimal nodal disease. Cases of patients with leukemic presentation and absence of nodal involvement represent *leukemic non-nodal variant* of disease, which is recently recognized in the updated World Health Organization (WHO) classification. While conventional MCL follows an aggressive clinical course, frequently characterized by relapses after initial treatment response, leukemic non-nodal MCL is associated with more indolent clinical course. The disease is

[2] Warburg effect: Increased LDH as a result of altered metabolism of cancer cells that depend on anaerobic respiration as the main source of energy, This results in production of lactate form glucose even under oxygen sufficient environment.

Table 2.1 Two subgroups of MCL

	Conventional MCL	Leukemic non-nodal MCL
Clinical presentation	Lymphadenopathy Extranodal	Leukemic Splenomegaly
Cell of origin	Naïve B cell	Memory B cell
Morphology	Classic/blastoid	Small cell/plasma cell differentiation
Phenotype	Majority CD5+, majority CD200-, SOX11+	Less than 50% CD5+, majority CD200+, SOX11-
Chromosomal instability	High	Low
Somatic mutations Major differences	ATM+++ CCND1 + CDKN2A del + KMTD2/MLL2 +	ATM (not reported) CCND1 ++++ CDKN2A del (not reported) KMTD2/MLL2 (not reported)
Somatic mutations seen in both entities	TP53 + BIRC3 + NOTCH1/ NOTCH2 (+)	TP53 + BIRC3 + NOTCH1/ NOTCH2 +
Clinical course	Aggressive	Indolent

Adapted from Puente et al. [33]

stable and asymptomatic for long periods, and tumor cells mainly involve peripheral blood and spleen but not lymph nodes in early stages, contrary to the patients diagnosed with conventional MCL who still have one of the worst clinical outcomes of all patients suffering from B-cell lymphomas. The neoplastic cells in leukemic non-nodal mantle cell lymphoma are most likely to be smaller and resemble CLL cells with mature chromatin in contrast to large leukemic cells with fine chromatin and nucleoli in the blastoid variant. Currently two distinct molecular groups and pathways are recognized that parallel differences in clinical presentation and course (See below and Table 2.1) [24–35].

Cell of Origin and Ontogeny

Most MCL cases are postulated to derive from naïve pre-germinal center B cells of the mantle zone, while a subset of MCL may originate from marginal zone or peripheral blood memory B cells. Historically, naive B cells have been considered the normal counterpart to MCL tumor cells based on IgM/IgD and CD5 expression by the tumor cells, their topographic distribution in the mantle zones, and early descriptions of the predominant use of unmutated immunoglobulin heavy chain variable region (*IgVH*) genes [33–37]. Somatic mutations in the immunoglobulin heavy chain variable region (*IgVH*) genes usually serve as a marker of germinal center transit and are not observed in naïve (not exposed to the stimulating antigen) cells. However, several studies have found somatic hypermutation in approximately 15–40% of MCL cases. *IGHV3-21* and *IGHV4-34* usage was significantly overrepresented in MCLs in these studies. The *VH3-21* MCL cases were highly associated

with expression of the lambda light chain, with selected use of *IGLV3-19 gene*, suggesting that these tumor cells are stimulated by an antigen recognized by immunoglobulin formed from these heavy and light chains. These findings suggest that at least in a subset of MCL cases, antigen drive is part of oncogenic process [26, 37–39, 40].

Pathobiology

Initial Oncogenic Events

Biologically, the chromosomal translocation t(11;14) is considered the genetic hallmark and primary oncogenic event in MCL [1, 16, 33, 36, 41–47]. The t(11;14)(q13;q32) translocation juxtaposes the proto-oncogene *CCND1* at 11q13 to the immunoglobulin heavy chain joining region (*IGH*) at chromosome 14q32. The major translocation cluster (MTC) was identified within around 80 base pairs region on chromosome 11 and in the 5′ area of one of the immunoglobulin JH regions on chromosome 14. Thirty percent to fifty-five percent rearrangements occur within this small region, while 10–20% cases have breakpoints in other distal regions. The small size of this region makes it possible to use PCR techniques for its detection [38].

This translocation forces the constitutive overexpression of cyclin D1 due to IGH promoter/enhancer. *Cyclin D1* gene was first identified in parathyroid adenomas with inversion 11, inv(11)(p15;q13). It was considered candidate oncogene and was designated PRAD-1. PRAD-1 sequence was highly homologous to cyclin family of proteins controlling cell cycle progression, and it was recognized as a novel member of cyclin D1 family and was renamed as cyclin D1 (CCND1) [48, 49].

CCND1 gene (*B-cell lymphoma 1 – BCL1, PRAD1*) encodes cyclin D1 protein, which is not detected in normal B lymphocytes [42]. Cyclin D1 is required for progression through the G1 phase of the cell cycle. Cyclin D1 binds to the cyclin-dependent kinases 4 and 6 (CDK4 and CDK6), forming a complex that phosphorylates and inactivates the retinoblastoma (Rb) protein leading to release of E2F transcription factor. This in turn leads to transcription of genes required for entry into the S phase. Inactive pRb allows cell cycle progression through the G1/S transition and allows for DNA synthesis. In most cases of MCL, retinoblastoma protein appears normal in structure and expression level with the exception of blastoid variants with high proliferation where pRb was shown to be hyperphosphorylated. E2F is frequently overexpressed in MCL. Overall, this further supports the role of cyclin D1 in suppressing anti-oncogenic effect of retinoblastoma protein [50–54].

Cyclin D1 may also interfere with the control of late G1 phase and G1- to S-phase transition. Normally this step is regulated by the cyclin E-CDK2 complex and the cyclin kinase inhibitor $p27^{Kip1}$. Cyclin D1-CDK4 also enables the activation of cyclin E-CDK2 complex by sequestering Cip/Kip family CDK inhibitory proteins p21 and p27, allowing entry into the S phase (Fig. 2.2) [54–56].

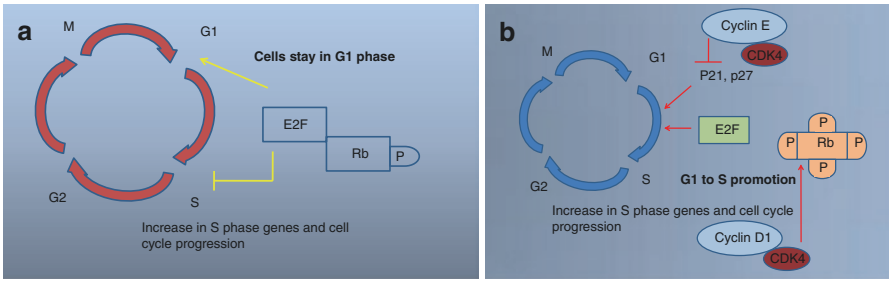

Fig. 2.2 (**a**) Cyclin D1 role in cell cycle regulation. Rb binds E2F transcription factor, thus preventing S-phase genes from activation and keep cells from G1 to S phase entry. (**b**) Cyclin D1 mediates its oncogenic effect through its binding to CDK4, which promotes pRb phosphorylation, E2F release, and subsequent promotion of the G1-/S-phase transition, cell cycle progression, and cell proliferation

In non-Hodgkin's lymphomas other than MCL, $p27^{Kip1}$ protein expression is inversely related to the tumor's proliferation activity as protein undergoes degradation by the proteasome pathway after sequestration induced by the overexpressed cyclin D1. Expression of p27Kip1 by immunohistochemistry is not usually seen in conventional MCL; however, its expression is unexpectedly observed in blastoid variants. This phenomenon is not clearly understood at present [55].

Additional mechanisms to increase levels of cyclin D1 in the MCL cells were described, such as amplification of the translocated allele or secondary rearrangements and mutations involving the three untranslated regions that generate more stable CCND1 transcripts and increased stability of the protein [36, 56].

Besides these mechanisms, cyclin D1 may have an important oncogenic potential independent of its catalytic function by acting as a transcriptional modulator of multiple genes involved in DNA repair and transcriptional regulation [33, 36, 37].

The t(11;14) translocation and subsequent cyclin D1 overexpression occur in the pre-B cells. This initial event is common between two distinct subtypes of the disease (*conventional* and more indolent (*non-nodal, leukemic*), but they differ in the expression of another important oncogenic element in MCL-SOX11 (Fig. 2.3, Table 2.1). SOX11 is a transcription factor that is expressed in the conventional MCL cells but not in B lymphocytes of more indolent MCL subtype. It is not expressed in normal B-lymphoid cells, but its expression was demonstrated in other B-cell neoplasms. The precise mechanism of SOX11 overexpression is not yet understood, but an activation mechanism through a three-dimensional interaction of a distant enhancer with the SOX11 promoter was recently suggested. SOX11 promotes tumor cell growth and regulates B-cell differentiation, cell proliferation, and tumor cell microenvironment. Furthermore, SOX11 induces expression of PAX5, a master regulator of B-cell differentiation. As PAX5 downregulation is required for plasma cell development, increased expression of PAX5 blocks B-cell differentiation process contributing to tumorigenesis. Downregulating BCL6 expression, SOX11 also prevents cells from entering the germinal center. Additionally, SOX11 regulates interactions of MCL cells with the microenvironment-promoting

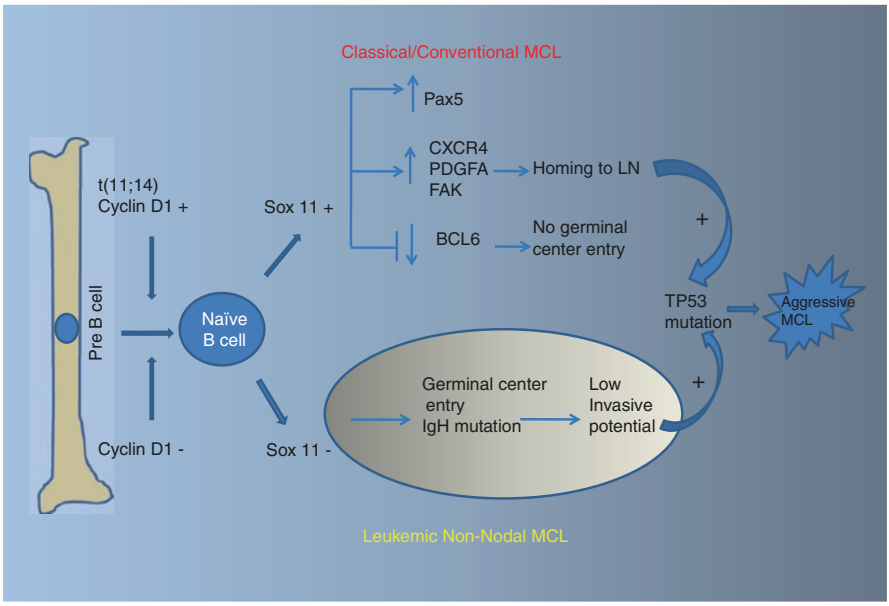

Fig. 2.3 MCL pathogenesis and molecular subtypes. MCL primary oncogenic event is the t(11;14) leading to CCND1 overexpression. The differential expression of SOX11 seems to define two subtypes. SOX11 expression represses BCL6 that may prevent cells from entering the germinal center. These cells carry unmutated IGHV and are genetically unstable. SOX11 may block the terminal B-cell differentiation of cells by forcing PAX5 expression and promote tumor cell growth, migration, and homing to lymph nodes via activation of the CXCR4/FAK/PI3K/AKT axis. SOX11-negative MCL cells enter the germinal center; they appear genetically stable. The SOX11-negative tumor cells have very low invasive potential and remain in the blood as leukemic disease and frequently discovered incidentally. Both MCL subtypes may acquire additional genetic events such as TP53 mutations that promote progression to aggressive variants

angiogenesis, MCL migration, and stromal-mediated drug resistance through the induction of platelet-derived growth factor a (PDGFA) and through direct regulation of CXCR4 and FAK expression and focal adhesion kinase (FAK)/PI3K/AKT pathway activation. These mechanisms are consistent with the extensive nodal dissemination and more aggressive behavior of conventional SOX11 MCL [33, 57–63].

There are few MCLs that lack the translocation t(11;14) and do not overexpress cyclin D1. Instead, these cases seem to frequently express higher levels of cyclin D2 or cyclin D3. In roughly 55% of these lymphomas, the cyclin D2 locus is rearranged, emphasizing the importance of cyclin D2 overexpression. These lymphomas resemble MCL by morphology and phenotype and yet lack BCl1 and the t(11;14). They are similar to MCL by gene expression profiling studies. Cyclin d2 and cyclin d3 expression by IHC is not useful as this is expressed by other low-grade lymphomas. They harbor translocation of cyclin d2 gene on chromosome 12 with heavy and light chain genes on chromosomes 14, 2, and 22, respectively. They express SOX11 transcription factor uniformly and hence are valuable in recognizing cyclin d1-negative cases of MCL.

Cyclin D1 expression alone is not sufficient for the transformation of normal B lymphocytes, as was shown in transgenic mice models that overexpress cyclin D1 alone does not develop lymphoma. In addition to cyclin D1 expression and its role in cell cycle dysregulation, MCL tumors demonstrate a decreased response to DNA damage and enhanced cell survival (impaired apoptosis) [59, 64–67].

In addition to cyclin D1 deregulation as the primary oncogenic event, genetic and molecular studies have identified other alterations involving the cell cycle machinery, the cellular response to DNA damage, and cell survival pathways that contribute to the disease progression (Fig. 2.4) [16, 33, 38, 68, 69].

Genome-wide studies have identified genes that are recurrently mutated or dysregulated in MCL besides *CCND1*. The most common secondary alteration is the mutation of ATM (42–55%), usually associated with 11q deletions, followed by mutation/deletion of TP53 (10–25%), reflecting the importance of DNA repair in the pathogenesis of MCL. In a subset of cases of MCL, the gene that encodes the NOTCH1 receptor is hypomethylated or has a gain-of-function mutation, suggesting that NOTCH signaling has an oncogenic role in some cases of MCL. Alterations in genes involved in apoptosis (*BIRC3*, *TLR2*), chromatin modification (*WHSC1*, *MLL2*, *MEF2B*), as well as the gene encoding the NOTCH2 receptor. *TP53* deletion and even more so mutation of *TP53* is associated with poor response to therapy and poor survival outcomes. The distribution of MCL driver genes differs considerably

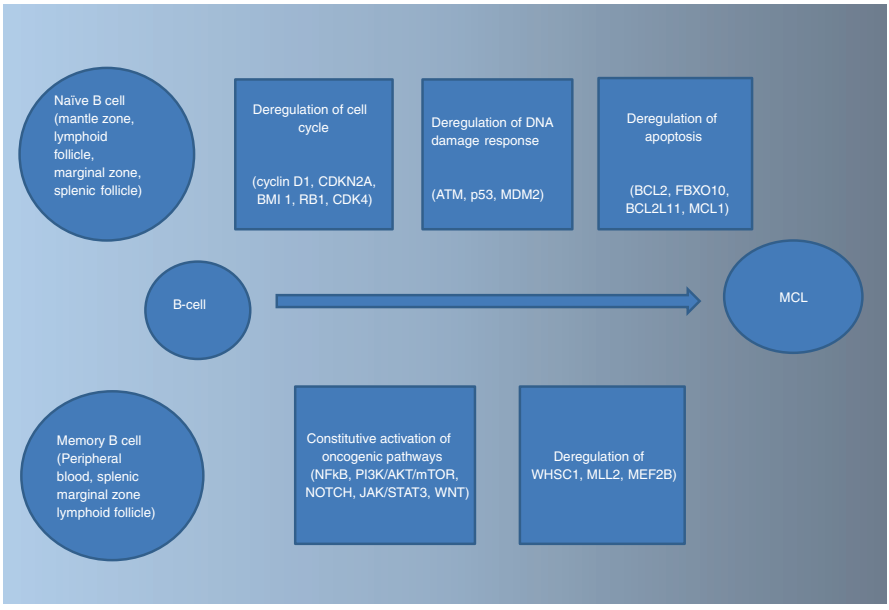

Fig. 2.4 Molecular pathways contributing to MCL pathogenesis. Diverse molecular events are involved in the pathogenesis of MCL. Adding to the initiating translocation t(11;14), deregulation of cell cycle, DNA damage repair, apoptosis and chromatin modifiers, as well as upregulation of various oncogenic pathways play crucial roles in MCL lymphomagenesis

between SOX11-positive and SOX11-negative tumors. ATM, CDKN2A, and chromatin-remodeling genes are almost exclusively altered in SOX11-positive cases, whereas TLR2 mutations appeared only in SOX11-negative tumors. Mutations in TP53, NOTCH2, and BIRC3 are demonstrated in both MCL subtypes [33, 69, 70].

Impaired DNA Damage Response

Some cases of MCL appear to have an impaired response to DNA damage, which results in increased chromosomal instability. This impaired response is largely due to defects in the *TP53* and ataxia-telangiectasia-mutated (*ATM*) genes. In addition, the cell cycle checkpoint kinases (CHK) 1 and 2 are downregulated in some MCL cases [33, 70].

TP53 Mutations of *TP53* are seen in 10–25% of MCL cases and are associated with more aggressive histology and shorter survival [71]. The *TP53* gene, located at 17p13.1, encodes a tumor suppressor protein that binds DNA and activates transcription of growth-inhibitory genes. It acts by regulating transcription of numerous downstream target genes involved in cell cycle arrest, apoptosis, DNA repair, senescence, and metabolism as a transcription factor and by being directly recruited to the mitochondria and inducing apoptosis independent of its function as a transcription factor [72, 73].

Under normal physiological conditions, p53 expression is maintained at a low level, regulated via degradation by its E3 ubiquitin ligases, MDM2, Pirh2, and COP1. However, under stressful conditions p53 is stabilized by posttranslational phosphorylation and acetylation. These stressors can be of different types such as double DNA-strand breaks in chromosomal DNA induced by ionized radiation. Another example is single-stranded DNA that develops at stalled replication forks due to base alteration induced by chemotherapeutic drugs and UV radiation. Aberrant growth signals that deregulate pRb-E2F cell cycle control pathway are additional mechanisms of stress. Once stabilized, TP53 facilitates variety of growth-limiting responses, including cell cycle arrest, apoptosis, senescence, and differentiation. When p53 activity is lost by gene deletion or mutations, normal cells lose the abilities to control their growth and death, leading to immortalization and ultimately cancer [74, 75] (Fig. 2.5).

Downregulation of *TP53* expression or expression of mutant *TP53* products results in a loss of the normal growth-limiting activities of this gene. Interestingly, mutation of *TP53* has been more strongly associated with poor outcome than its deletion, raising the possibility that gain-of-function mutations are involved [76].

Mutations in the *p53* gene occur mainly in the DNA-binding domain with the majority of them being missense mutations, resulting in loss of function as a transcription factor and accumulation of dysfunctional p53 protein in tumors [76]. There are two classes of mutants: DNA contact (class I) where mutations involve

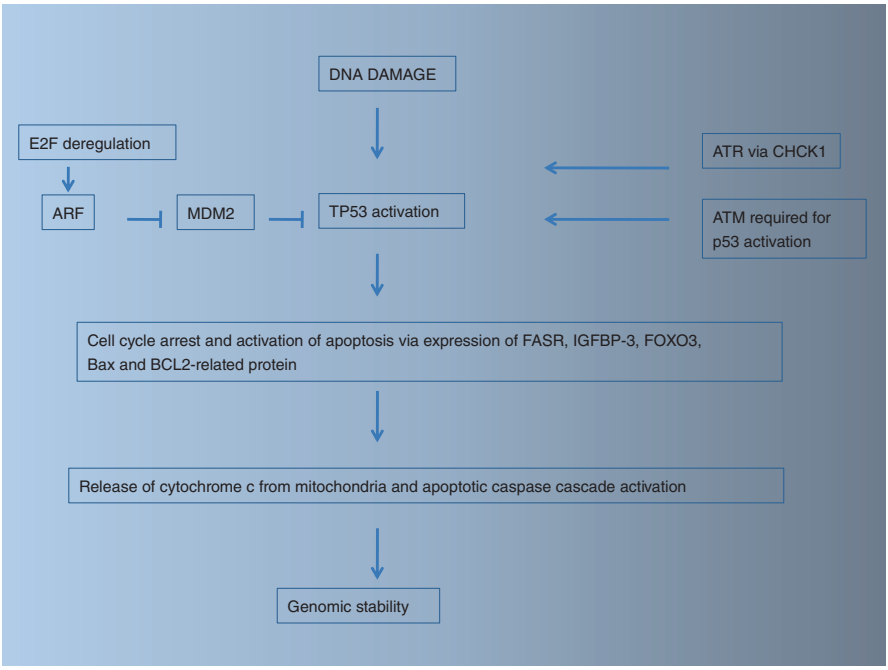

Fig. 2.5 P53 response to DNA damage pathway. MDM2 is a negative regulator of p53. Upon DNA damage ARF promotes MDM2 degradation and p53 stabilization and activation, along with ATR and ATM. p53 utilizes multiple signaling pathways to activate the apoptotic program, such as increase FAS receptor on the cell surface and sensitizing the cell to any Fas ligand in the extracellular space, inducing IGF-binding protein-3 which leads to activation of Bad, FOXO3, and IkB, and increase expression of FOXO3-, Bad-, and BCL2-related protein which causes the release of cytochrome c and caspase cascade activation leading to cell death

amino acids directly binding to the p53-responsive element in DNA (e.g., $p53^{R273H}$ and $p53^{R280K}$) and conformational (class II) where mutations lead to structural changes of p53 protein to diminish its DNA-binding activity.

Both mutant types also demonstrate the dominant-negative (DN) activity by heterooligomerizing with and deactivating wild-type p53. Some mutations may lead to oncogenic gain-of-function (GOF) activities, such as enhanced tumor progression, metastatic potential, and drug resistance, when overexpressed even in cells lacking wild-type p53. Thus, mutant p53 can function as an oncogene and greatly contribute to malignant potential of cancer cells. In a subset of MCLs, inactivation of p53 can also be mediated through overexpression of MDM2 that promotes its proteasome-dependent degradation [76–79].

ATM Gene Deletion of 11q22-23 or point mutations in the ataxia-telangiectasia-mutated (*ATM*) tumor suppressor gene is seen in approximately one-third to one-half of MCL cases. ATM is involved in the detection of DNA damage and plays an important role in the regulation of cell cycle progression. *ATM* mutations and *TP53* mutations are seen together in only 10% of cases [70].

The *ATM* gene was discovered in 1995 in patients with ataxia-telangiectasia (A-T) syndrome [80, 81]. It is located on chromosome 11q 22–23 (11q22-q23) and encodes a PI3K-related serine/threonine protein kinase (PIKK). PIKK plays a central role in the repair of DNA double-strand breaks (DSB), which can be, as was mentioned above, induced by ionizing radiation, chemotherapy drugs, or oxidative stress or occur during normal physiologic events like meiotic recombination or rearrangement of antibody genes during B-cell maturation [82, 83]. Repair of DSBs involves an extensive network of signals, including sensor/mediator proteins, downstream transducer protein kinases, and effector proteins. The MRE11-RAD50-NBS1 (MRN) complex is considered the primary sensor/mediator of DSBs [84]. It forms a physical bridge spanning the DSB ends, takes part in DSB end resection, and recruits ATM at DSB sites (Fig. 2.6). ATM behaves as a transducer in the DSB repair process, connecting with other sensor proteins, such 53BP1 (p53-binding

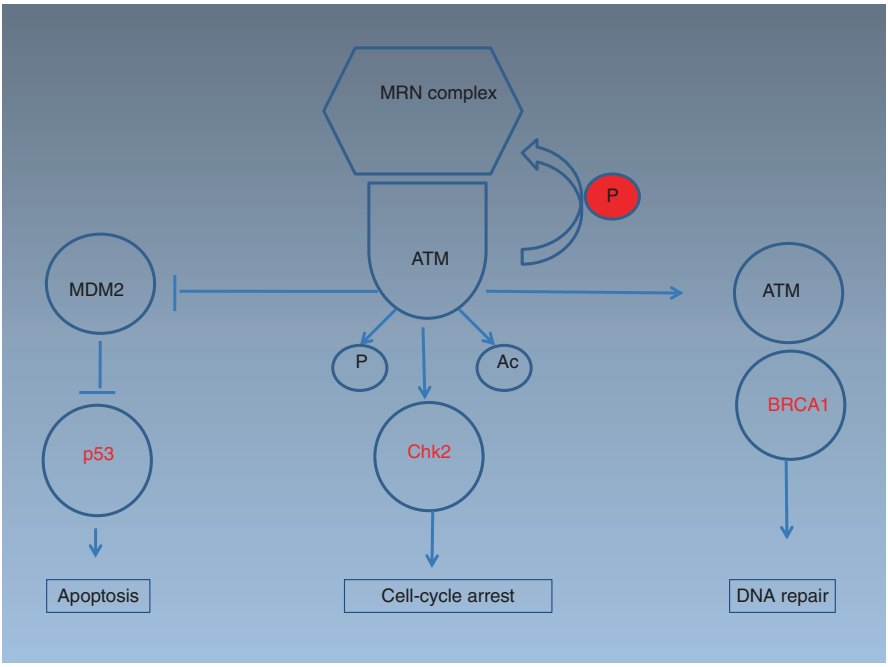

Fig. 2.6 ATM pathway. ATM in repair of double-stranded DNA breaks. MRN complex finds the breaks and recruits ATM. ATM dissociated into active monomers; it is autophosphorylated and acetylated at the recruitment site. After ATM phosphorylates sensor proteins and protein kinases and activates checkpoint kinase 2 (CHK2), p53 and BRCA lead to cell cycle checkpoint control, induction of apoptosis, and DNA repair. ATM inactivation in MCL leads to defects in ATM-dependent phosphorylation of its substrates (in red) and contributes to the genetic instability and defective cell cycle control observed in MCL cells. ATM inactivation in tumor cells thus may act synergistically with cyclin D1 overexpression to override cell cycle checkpoint controls and thereby promote the accumulation of additional genomic aberrations during tumorigenic development

protein) and BRCA1 (breast cancer type 1). ATM becomes catalytically activated at the site of DSBs by dissociating from homodimers into active monomers, autophosphorylation of ATM at Ser1981 and other sites, and acetylation [85–87]. Activated ATM turns on phosphorylation and subsequent activation of many downstream effectors, including ones involved in cell cycle checkpoint arrest (Chk1 and Chk2), DNA repair (BRCA1 and RAD51), and apoptosis (p53) [75, 88].

ATM mutations observed in approximately 45% of mantle cell lymphoma cases; such mutations generally are truncating mutations or missense mutations within the region of the gene encoding the PI3K domain [89]. The high frequency of *ATM* mutations has been linked to ATM expression in naive B cells in the mantle zone. ATM mutations are seen in cases with high chromosomal instability where aberrant reinitiation of DNA replication during the S phase and decrease in the expression of CHK1 and CHK2, 2 serine-threonine kinases downstream of S-phase checkpoints have been identified [90].

Enhanced Cell Survival Microarray studies have suggested that MCL cases display disturbances of pathways associated with apoptosis. Specifically, MCL cells appear to avoid programmed cell death (apoptosis) by the expression of B-cell lymphoma 2 (BCL2), upregulation of the PI-3 kinase/AKT pro-survival signaling pathway, and activation of nuclear factor-kB (NF-kB) [33, 36, 69].

B-cell leukemia/lymphoma 2 (BCL-2) family proteins are key regulators of apoptosis [91]. BCL2 family members regulate cell survival and can either promote or inhibit apoptosis. They share regions of homology and can be classified into either antiapoptotic members, multidomain proapoptotic, or BH3-only proapoptotic members (Fig. 2.7).

BCL2 is an oncogene that blocks programmed cell death, leading to prolonged cell survival [75]. BCL2 overexpression is also common in other B-cell lymphomas. The BCL-2 pathway is deregulated in MCL; high-level copy number gains of the *BCL2* locus at 18q21.3 are frequent [92]. High MCL1 expression is common in tumors that are more aggressive and is increased by AKT/mTOR signaling. BCL-XL overexpression has been linked to constitutive activation of the NF-kb pathway [75, 91, 93, 94].

In normal B cells, PI3K functions as a transducer of BCR signaling that regulates proliferation, differentiation, apoptosis, and survival. Gene expression profiling implicated the PI3K/AKT pathway in the pathogenesis of MCL, and several key components of the PI3K/AKT/mTOR pathway are activated in MCL. Constitutive activation of AKT was found in most blastoid and many classic MCL tumors and was associated with the phosphorylation of downstream targets, including MDM2, Bad, and p27. Furthermore, AKT mediated activation of mTOR, and its downstream targets S6K and eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) can increase translation of key proteins.

The PI-3 kinase (PI3K)/AKT signaling pathway promotes cell growth, cell survival, and tumorigenesis by inactivating several proteins including CDKI1B (p27) BAD (a member of the BCL2 family) and FOXO1, a transcription factor that regulates the expression of proapoptotic genes (Fig. 2.8). The PI3K/AKT pathway also

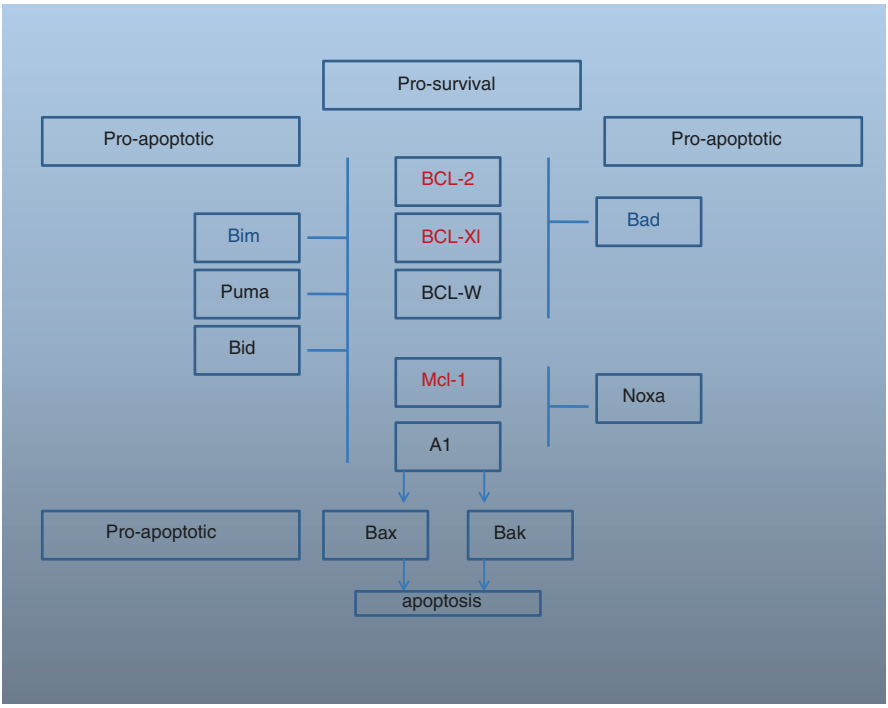

Fig. 2.7 Bcl-2 family members and antiapoptotic alterations in MCL. Bcl2 family consists of antagonistic partners. In red are family members commonly overexpressed in MCL, in blue are frequently deleted or inhibited with overall antiapoptotic advantage in MCL cells

increases the levels of nuclear factor-kappa B and mammalian target of rapamycin (mTOR), resulting in a decrease in functional TP53 and subsequent cell survival. Bruton tyrosine kinase (BTK) functions downstream of the membrane-bound B-cell receptor (BCR) and links constitutive BCR signaling to PI3K/AKT activation and nuclear factor-kappa B activation, consistent with the view that these pathways are critical for MCL growth and survival [95–97]. Several mechanisms may cause constitutive activation of AKT, including activation of upstream kinases such as SYK, and amplification of *PI3KCA*, the gene encoding the catalytic subunit p110 [97]. Loss of PTEN, a phosphatase that turns the PI3K pathway off, is another recurrent alteration in MCL and may be the result of mutations, deletions, or promoter methylation [96]. PTEN can also be inactivated by phosphorylation at serine 380 and threonine 382/383, which has been found in MCL cases with constitutively active AKT [75, 98].

Nuclear Factor-Kappa B (NF-kB) Pathway Nuclear factor-kappa B (NF-kB) polypeptides represent a family of transcription factors that have important roles in regulation of normal immune function and are often dysregulated in lymphoid malignancies. It consists of p65 (RelA), c-Rel, RelB, p50/p105 (NF-kB1), and p52/p100 (NF-kB2) [75]. They activate the transcription of genes involved in survival,

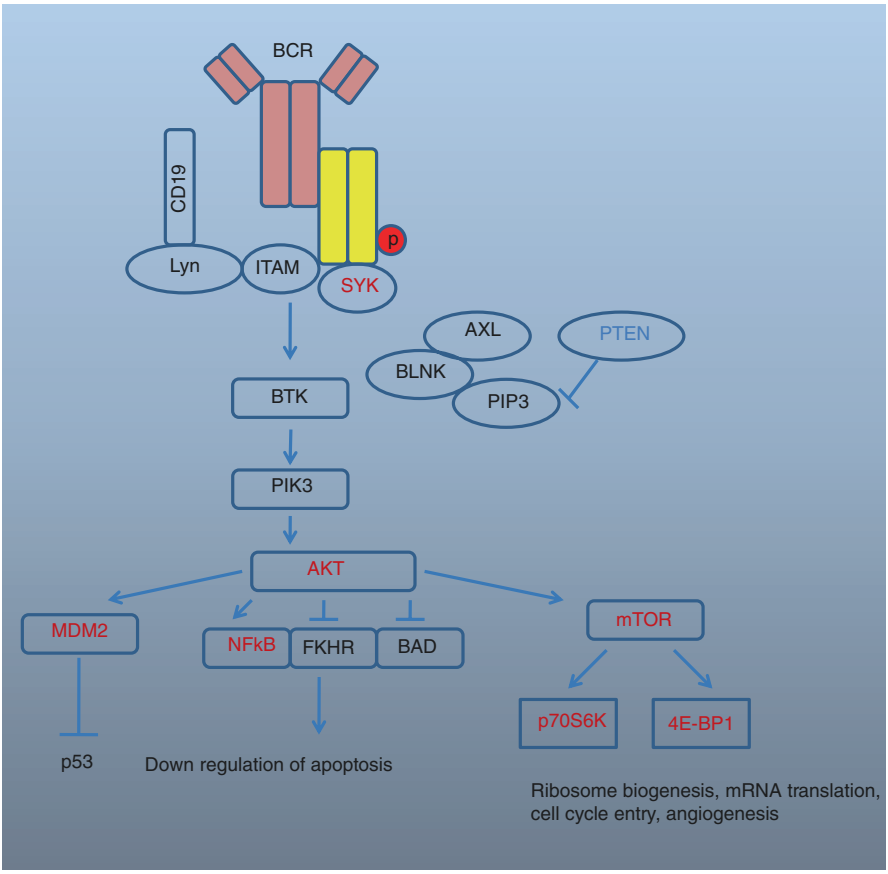

Fig. 2.8 PI3K/AKT/mTOR pathway. PI3K functions as a transducer of BCR signaling and can be activated by SYK-dependent phosphorylation of CD19 and B-cell PI3K adaptor protein (BCAP). PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to generate the second messenger, phosphatidylinositol-3,4,5-trisphosphate (PIP3). This process is negatively regulated by PTEN. PI3K then phosphorylates the serine/threonine kinase AKT with subsequent activation of mTOR, MDM2, and NF-kB (by activation of IKK) contributing to MCL pathogenesis. In blue inactivated or downregulated molecules, in red activated or overexpressed molecules

proliferation, and apoptosis [99]. Upon activation through the classical/canonical or the alternative/noncanonical pathway, the NF-kB members are released from their inhibitors and subsequently translocate to the nucleus to bind to their target genes [100]. Constitutive activation of both the classical and the alternative NF-kB pathway has been detected in MCL [101, 102]. NF-kB target genes highly expressed in MCL include the antiapoptotic proteins BCL-2, BCL-XL, XIAP, and cFLIP [37, 101, 103].

NF-kB signaling can also drive transcription of the TNF family member BAFF/BLyS, a potent B-cell survival factor. BAFF in turn stimulates both canonical and

alternative NF-kB pathways, creating a positive feedback loop that could contribute to tumor cell survival [104].

BAFF receptor was found in the nucleus of MCL cells, where it participated in histone H3 phosphorylation, leading to an increase transcription of genes that promote cell survival and proliferation [105]. Inactivation via genomic deletions, mutations, and increased promoter methylation of a negative regulator, ubiquitin-editing enzyme *TNFAIP3*/A20, also contributes to a constitutive activation of NF-kB signaling [106] (Fig. 2.9).

NOTCH Pathway NOTCH is a transmembrane protein that functions as a ligand-activated transcription factor. NOTCH pathway is one of the most evolutionarily conserved signaling pathway that regulates important cell fate decisions during embryonic development. In adult tissues, NOTCH-mediated signals are important regulators in the maintenance of self-renewal, contributing, for example, to myogenesis, neurogenesis, and lymphocyte development [75, 107].

NOTCH1and NOTCH2 mutations have been detected in MCL [108]. Approximately 10% of MCLs harbor somatically acquired NOTCH1 mutations [70]. The majority of recurrent mutations in *NOTCH1* are located in exon 34 that encodes the PEST domain. PEST domain is a C-terminal region of intracellular

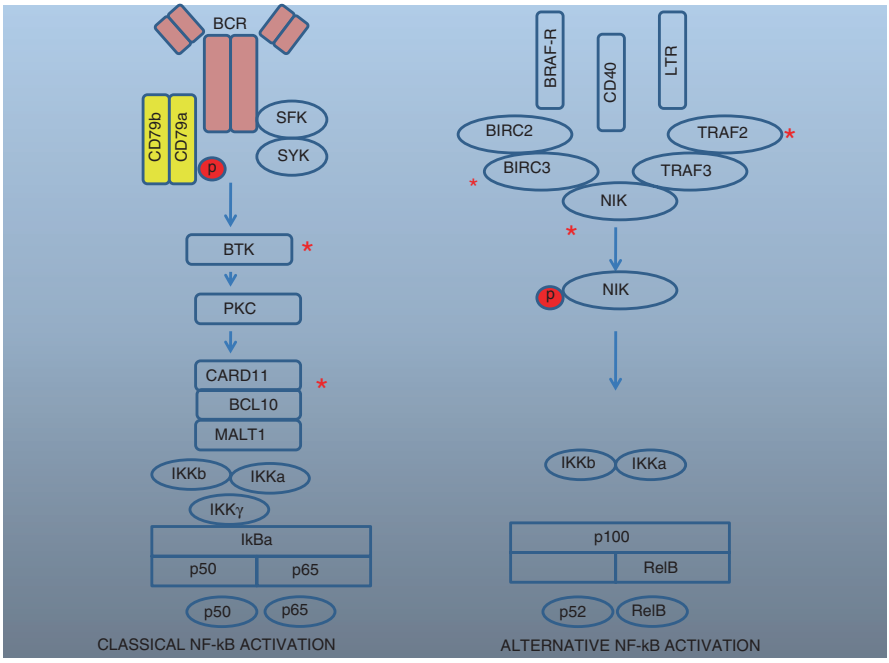

Fig. 2.9 Chronic active BCR and NF-kB signaling in MCL. Constitutive activation of both NF-kB signaling pathways is described in MCL and was shown to have an important role in MCL contributing to MCL cells survival and proliferation. * indicates mutation detected in MCL cells

portion of the NOTCH1 molecule rich in proline, glutamate, serine, and threonine and regulates protein stability and degradation. These mutations result in an abnormal overactivation of NOTCH1 signaling pathway due to impaired degradation of its active intracellular component [109].

These mutations are predominantly caused by truncation or small frameshifting indels [70, 109]. Mutations in *NOTCH1*are associated with a shorter overall survival, suggesting a negative prognostic role for *NOTCH1* in MCL. It was recently shown that NOTCH signaling regulates, directly or indirectly through MYC, a gene signature that maintains BCR signaling, RNA metabolism, and chromatin/transcriptional regulation [110, 111]. In addition, hypomethylation of NOTCH1 seems to represent an alternative mechanism of NOTCH activation [112].

NOTCH2 mutations occur in roughly 5% of MCLs [70]. These mutations are more frequent in blastoid and/or pleomorphic MCLs and are associated with a dismal prognosis.

NOTCH2 mutations are present as an alternative and are mutually exclusive to *NOTCH1* alterations in aggressive tumors. The mutation pattern is similar to that reported for *NOTCH1*, with the generation of a premature stop codon within the PEST domain. *NOTCH2*-mutated cases demonstrate an upregulation of different genes involved in cell cycle and metabolic pathways, together with genes directly regulated by *NOTCH2* [70, 111] (Fig. 2.10).

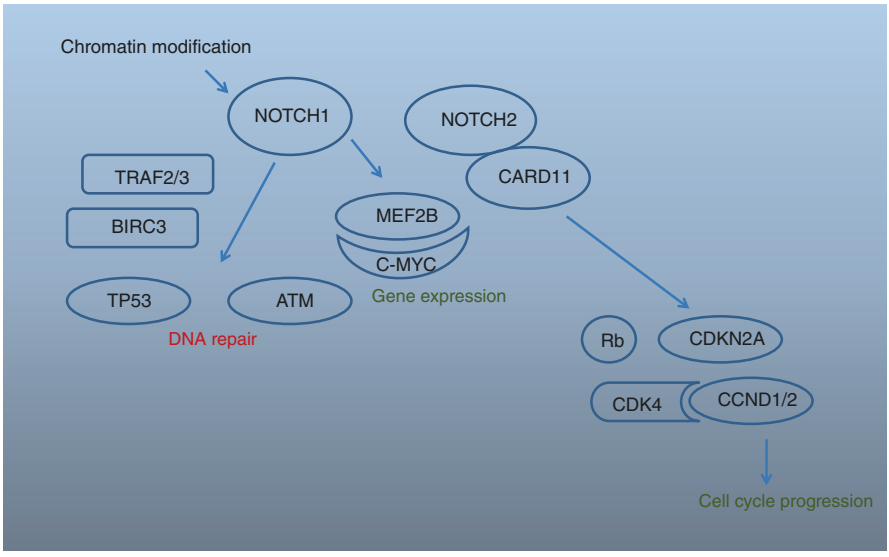

Fig. 2.10 NOTCH1 and NOTCH2 participate in regulation of DNA repair, gene expression, chromatin modification, and cell cycle in MCL. NOTCH1 along with BIRC3, and TRAF2/TRAF3 regulates TP53 and ATM and directly and indirectly activates C-MYC. NOTCH1 activation via mutations or chromatin modification leads to increased gene expression and impaired DNA repair. NOTCH2 recruits CARD11 upon activation and leads to downstream activation of cell cycle activators promoting cell proliferation

Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) pathway – *the JAK/STAT3* pathway mediates cell survival and cell proliferation and is activated by extracellular ligands such as interleukins (IL) or interferons. STAT3 is the signal transducer of IL-10, and STAT3 is activated by phosphorylation [75, 113].

Ligand binding induces autophosphorylation of JAK that in turn phosphorylates and activates STAT3. Activated STAT3 translocates to the nucleus to alter transcription. Interleukin (IL)-10 was shown to increase the proliferative activity of MCL cells. Phosphorylated STAT3 has been detected in primary MCL tumors and seems to be induced by BCR engagement and/or an autocrine IL-6 and/or IL-10 secretion loop [114, 115]. Additional evidence for the important role of JAK/STAT signaling in MCL came from a recent sequencing analysis of suppressor of cytokine signaling 1 (SOCS1), an inhibitor of the JAK/STAT pathway. SOCS1 mutations were detectable in primary MCL cells [37, 116].

WNT Pathway The WNT pathway is involved in cell proliferation and survival. Signaling through this pathway can be divided into canonical/b-catenin-dependent and noncanonical/b-catenin-independent noncanonical signaling [75]. Gene expression profiling studies suggested a role of canonical WNT signaling in the pathogenesis of MCL. WNT pathway activation stabilizes beta-catenin, allowing it to translocate into the nucleus and form a transcription complex that upregulates the expression of multiple target genes, including *CCND1*.

Accumulation of b-catenin is seen in the nucleus of MCL cells, and inhibition of WNT signaling led to reduced proliferation and apoptosis of MCL cells, supporting a role of WNT pathway in MCL pathogenesis [117–120].

BCR Signaling The BCR is composed of an IgM and IgD immunoglobulin and the heterodimer CD79a/CD79b. BCR stimulation activates a molecular complex, including Bruton tyrosine kinase (BTK), phospholipase Cg2 (PLCG2), and the phosphoinositide 3-kinase (PI3K) with downstream signals promoting cell survival and proliferation. BCR stimulation also triggers the release of chemokines and increased migration of the cells. The signaling response to BCR engagement is variable among cases ranging from intense to anergy, an unresponsive status of the B cell induced by chronic antigen stimulation in the absence of T-cell cooperation. Activation of mucosa-associated lymphoid tissue 1 (MALT1)-driven MYC signaling, autocrine secretion of IL-1b, tumor necrosis factor-a (TNF-a), CCL5, and stromal-induced FAK activation lead to MCL survival, proliferation, and drug resistance. The tissue microenvironment provides an essential niche for MCL. Interactions between MCL cells and the microenvironment are mediated by different chemokines, adhesion molecules, and their respective receptors that are similar to those used by CLL. BCR and NF-kB activation mainly occurs in lymph nodes, but some leukemic cases have this activation independent of the nodal microenvironment (Fig. 2.9) [33, 36, 37, 121–125].

Epigenetic Regulation of Tumor Biology

Epigenetic changes are relatively new field in the MCL biology. While acetylation of histones leads to an open chromatin conformation and enhances access of transcription factors to DNA, methylation of gene promoters can silence gene expression. Alterations in both acetylation and methylation have been shown in tumorigenesis. Several hypomethylated and thereby upregulated genes could play a pathogenic role, including *NOTCH1*, *CDK5*, and *HDAC1* [33, 37, 112, 126, 127].

Deregulation of chromatin modifiers – mutations in chromatin modifiers such as *WHSC1* encoding a H3K36 methyltransferase, *MLL2* encoding a H3K4 methyltransferase, and *MEF2B* encoding a transcriptional activator – occur in subsets of MCLs. *MLL2* harbors frequently monoallelic truncating and missense mutations that may lead to loss of function of this haploinsufficient tumor suppressor gene [70, 128].

Multistep Development and Progression in MCL

The presence of critical oncogenic players does not correspond to the tumor proliferation. It is not yet clear how exactly oncogenesis progresses and what are the required stimuli and mutation to warrant progression (Fig. 2.11). Cells carrying the t(11;14) translocation have been detected at very low levels in the peripheral blood of a number of healthy individuals (8%). These clones can persist for long periods, but it is not clear whether they all evolve into an overt lymphoma. The rather high frequency in healthy individuals and low prevalence of MCL suggest that most

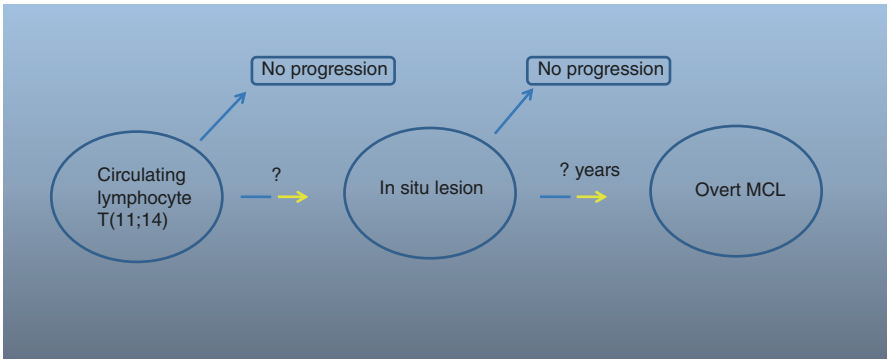

Fig. 2.11 Multistep progression of MCL (adapted from [35]). Clonal cells carrying the t(11;14) translocation may be detected in the peripheral blood of healthy individuals at very low levels. The risk of progression of these clones must be extremely low, if any. Cells expressing cyclin D1 and carrying the t(11;14) may be found in the mantle zone of lymphoid follicles in otherwise reactive tissues. Most of these lesions will not evolve into an overt lymphoma. It remains controversial whether in situ neoplasia is a required step in all lymphoma cases

clones bearing only the t(11;14) translocation will never transform into a malignant tumor. The incidence of MCL occurring simultaneously in the donor and recipient of allogenic bone marrow transplant years after transplant highlights long period of time for initial clones to progress to an overt lymphoma. Cells carrying the t(11;14) translocation and cyclin D1 overexpression have been occasionally found in the mantle zones of otherwise reactive lymphoid tissues in healthy individuals. These lesions have been referred to as "in situ MCLs" or "MCL-like cells," but their malignant potential seems very limited, and currently they are termed as *in situ mantle cell neoplasia*. The definition is the presence of cyclin D1-positive lymphoid cells with CCND1 rearrangements restricted to the mantle zones of otherwise hyperplastic-appearing lymphoid tissue. The cyclin D1-positive cells are typically seen in the inner mantle zone but may be rarely seen in the outer mantle zone and very rarely interfollicular. Compared with classic mantle cell lymphoma, this case is unlikely to be CD5 negative. The cases of in situ mantle cell neoplasia are rare and are usually identified as incidental finding sometimes associated with other lymphomas. These lesions usually have an indolent course and do not require therapy; however, follow-up is advised as rare cases may progress to overt lymphoma.

The in situ lesions were demonstrated in the lymph nodes that developed lymphoma years later. In situ lesion appears to be common step in both SOX11 positive and negative MCLs; however, it is not yet clear whether it is a mandatory step in all cases. In situ lesions were detected in patients in complete clinical remission suggesting that these in situ lesions may represent microenvironment niche to sustain resistance to treatment by tumor cells [2, 35, 128–132].

Morphology

The architectural and cytologic features of MCL have a broad spectrum as was pointed out in early morphologic descriptions of this lymphoma. Several architectural patterns and cytologic variants are currently recognized as distinct features of MCL in the current WHO classification [2, 16, 33, 37].

Architectural Patterns

When MCL presents as nodal involvement, normal lymph node architecture is effaced by a monomorphic tumor proliferation in either diffuse, mantle cell, or nodular patterns (Fig. 2.12, Table 2.2) [2, 16, 133].

Nodular pattern is usually vague, and transitional areas between nodular and diffuse pattern are usually seen. In rare cases, nodularity can be prominent raising consideration of follicular lymphoma in the differential diagnosis (rare follicular growth pattern). Some nodules may be solid, without evidence of residual germinal centers (representing malignant counterpart of primary follicles). Nodular pattern

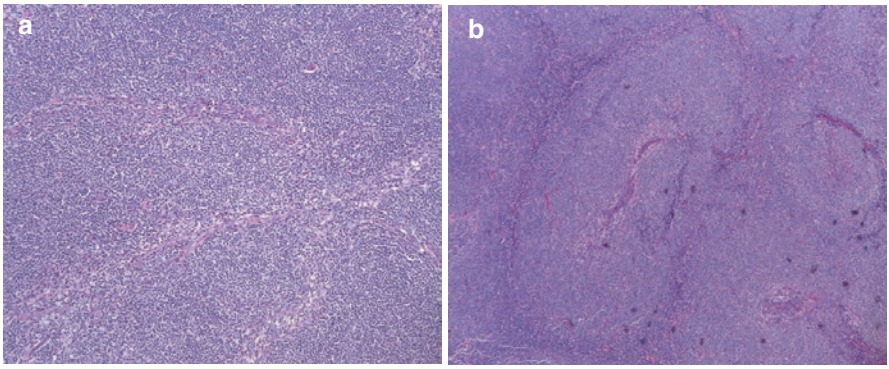

Fig. 2.12 MCL growth patterns. (**a**) Diffuse pattern. (**b**) Nodular pattern

Table 2.2 Mantle cell lymphoma pattern of growth

Diffuse pattern	Diffuse infiltration with lymph node architectural effacement
Mantle zone pattern	Neoplastic infiltration limited to the mantle zone surrounding reactive germinal centers
Nodular pattern	A vaguely nodular pattern, with obliteration of follicles and germinal centers

may also represent as infiltration and obliteration of the original germinal center by tumor cells. Cyclin D1, staining may identify initial infiltration or colonization of reactive germinal centers in some cases, as an early stage of a nodular pattern. Residual germinal centers can also be seen in tumors with a more diffuse pattern, although in these cases they may be identified only focally [4–6, 9, 15, 133].

The mantle zone pattern is characterized by expansion of the follicle mantle area by tumor cells surrounding a reactive germinal center, and it may be difficult to distinguish from follicular or mantle cell hyperplasias [134].

Cytologic Variants

Classic (conventional or typical) MCLs are characterized by a monotonous proliferation of small- to medium-sized lymphoid cells with scant cytoplasm, variably irregular nuclei, evenly distributed condensed chromatin, and inconspicuous nucleoli. Large cells with abundant cytoplasm or prominent nucleoli are rare or absent; when present, they may correspond to reactive centroblasts of residual germinal centers overrun by lymphoma cells.

Other variants as designated in the current WHO classification include small cell and marginal zone like [2, 16] (Figs. 2.13, 2.14, 2.15, and 2.16; Table 2.3). Some cases may show a predominance of small lymphocytes with rounded nuclei and more condensed chromatin. This so-called small-cell variant of MCL mimics

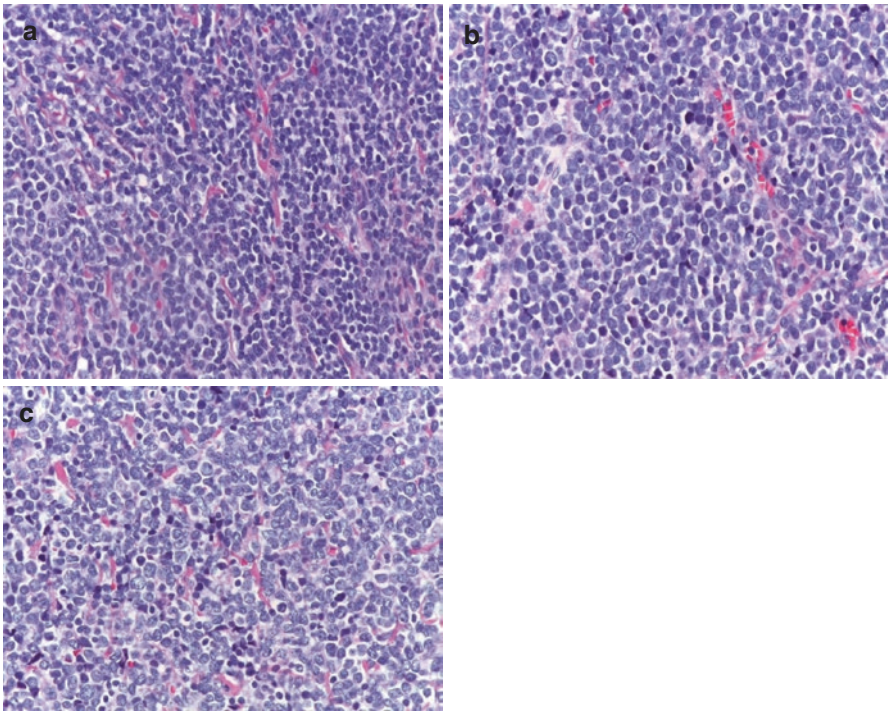

Fig. 2.13 Cytologic MCL variants in lymph node. (**a**) Classic/conventional variant (most common). (**b**) Blastoid variant. (**c**) Pleomorphic variant

chronic lymphocytic leukemia-small lymphocytic lymphoma (CLL/SLL) and has similar clinical behavior. The absence of proliferation centers (growth centers) or isolated prolymphocytes and paraimmunoblasts helps to distinguish this variant MCL from CLL.

Some cases may have a variable number of cells with more abundant pale cytoplasm, mimicking marginal zone or monocytoid B cells [135, 136]. While the nucleus of these cells may have a blastoid or classic morphology, the unusual pale abundant cytoplasm raises the possibility of marginal zone lymphomas or hairy cell leukemia. In some cases, these monocytoid-like cells may even reside in the marginal zone of lymphoid follicles, outside an apparently preserved mantle zone. CD5 and cyclin D1 positivity is crucial in the diagnosis of this variant [15].

Proliferative activity in classic and small-cell MCL varies from case to case but is usually lower than one to two mitoses per high-power field. However, some cases of conventional MCL may show a relatively high mitotic index, similar to the blastoid variants, and these patients may have an aggressive clinical course. Scattered epithelioid histiocytes with eosinophilic cytoplasm are relatively common, but well-formed microgranulomas are not usually seen. These histiocytes generally do not contain phagocytosed apoptotic bodies. In some cases, hyalinized small vessels may be seen scattered throughout the tumor [137, 138].

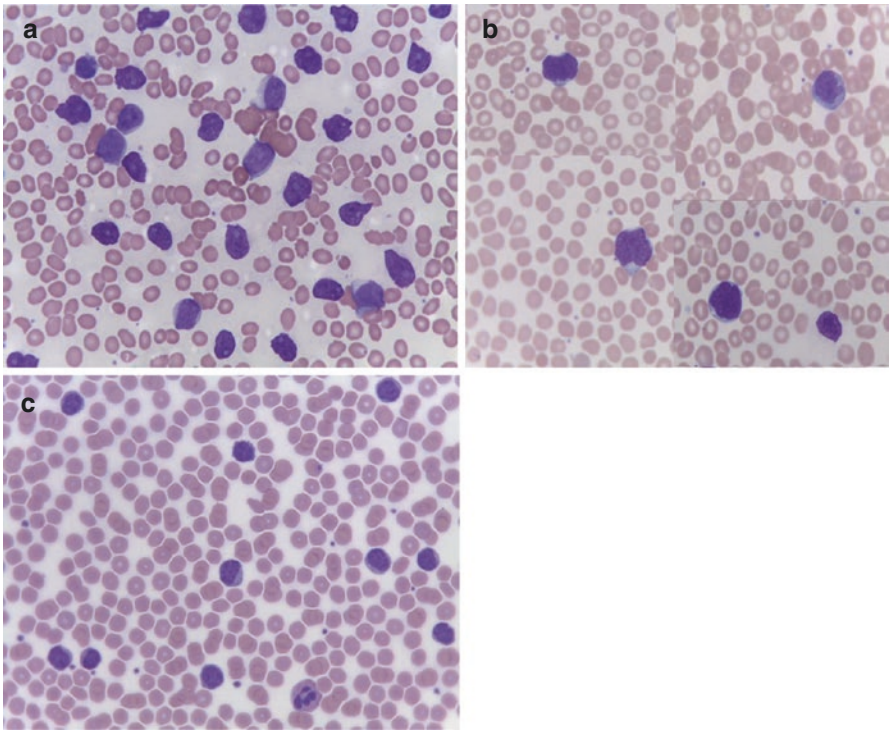

Fig. 2.14 Cytologic variants of MCL in peripheral blood. (**a**) Blastoid variant. (**b**) B. Pleomorphic variant (composite image). (**c**) Conventional variant

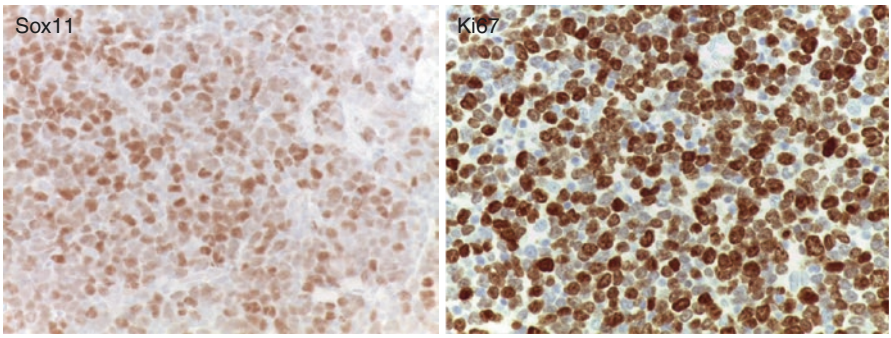

Fig. 2.15 Sox11 and high Ki-67 proliferation index in blastoid MCL (compared to lower Ki-67 index in conventional MCL, Fig. 2.1)

More aggressive variants of MCL may have a morphology that ranges from a monotonous population of cells resembling lymphoblasts (blastoid variant) to a more pleomorphic appearance with larger irregular cells resembling diffuse large B-cell lymphoma (pleomorphic variant) [137, 138]. These variants may represent the ends of a morphologic spectrum; transitional areas between these subtypes may

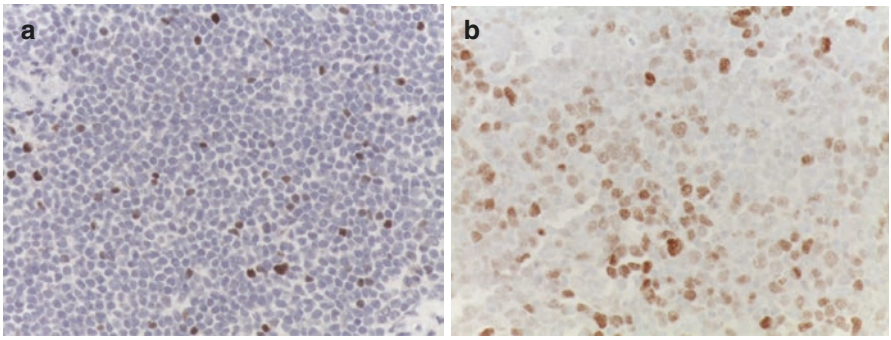

Fig. 2.16 C-MYC expression in conventional (**a**) and blastoid (**b**) MCL

Table 2.3 Variants of mantle cell lymphoma based on morphology

Classic	Small- to medium-sized lymphoid cells with slightly to markedly irregular nuclei, resembling centrocytes Moderately dispersed chromatin, inconspicuous nucleoli Scanty pale cytoplasm Monotonous cell populations Infrequent larger neoplastic cells Recognizable mitotic figures
Small cell	Small round lymphocytes, resembling CLL/SLL cells Dense, clumped chromatin
Blastoid	Intermediate-sized blasts, with a morphology between a centrocyte and a centroblast and mimicking ALL/B-LBL cells
Pleomorphic	Medium to large cells with large cleaved or oval nuclei Clumped or finely vesicular chromatic; prominent nucleoli Moderate amount of pale cytoplasm

be observed, and some tumors have mixed cytology, with areas of pleomorphic cells intermingled with others having a classic morphology. These cytologic variants have the same phenotype and genetic alterations, including 11q13 translocations and cyclin D1 overexpression, as classic MCL, indicating that they correspond to the same entity.

Blastoid MCL is characterized by a monotonous population of medium-sized lymphocytes with scant cytoplasm, rounded nuclei with finely dispersed chromatin, and inconspicuous nucleoli. These cases may resemble lymphoblastic lymphoma or nodal involvement by acute myeloid leukemias. The mitotic index is very high, with more than two to three mitoses per high-power field. Histiocytes with tingible bodies and a "starry-sky" pattern may be seen. MCLs with a more pleomorphic or large cell morphology were initially designated in the Kiel classification as *anaplastic centrocytic lymphomas* or *centroblastic lymphomas of the centrocytoid subtype* [139]. These tumors are composed of a more heterogeneous population of large cells with ovoid or irregular, cleaved nuclei; finely dispersed chromatin; and small, distinct nucleoli. The mitotic index is high but usually lower than in blastic cases. This pleomorphic variant may be difficult to distinguish from large cell lymphomas. However, the nuclear characteristics of cleaved contours, finely dispersed

chromatin, and discordance between the large nuclei and relatively small nucleoli may suggest a mantle cell origin. Ancillary studies, such as immunophenotyping, FISH for t(11;14) are mandatory in these cases to confirm the diagnosis [16].

Bone Marrow and Peripheral Blood

Bone marrow involvement is observed in 50–90% of patients and is detected more frequently in core biopsies than in aspirates. The pattern of infiltration may be nodular, interstitial, or paratrabecular, and in most cases, the combination of pattern is seen. Isolated paratrabecular aggregates are rare. In some cases, diffuse infiltration of the marrow may be observed. The degree of infiltration does not seem to correlate with the histologic variants of MCL identified in lymph node biopsies, architectural patterns, or patient survival [2, 16, 24, 140, 141].

Circulating peripheral blood cells in most MCLs usually show a mixture of small- to medium-sized cells with scant cytoplasm, prominent nuclear irregularities, and reticular chromatin. Some cells may have rounded nuclei, but the chromatin does not have the clumped appearance seen in CLL. Leukemic blastoid MCL may mimic acute leukemia, with medium to large cells, a high nuclear-to-cytoplasmic ratio, fine dispersed chromatin, and relatively small or inconspicuous nucleoli. Leukemic phase of the pleomorphic variant of MCL shows very large atypical cells and prominent nucleoli [2, 16, 142].

Spleen

Grossly, MCL in the spleen demonstrates micronodular pattern of growth occasionally associated with perivascular infiltration. Histologically, usually the enlarged white pulp nodules are seen with only variable involvement of red pulp. Cytologically the cells are rather monomorphic. Areas of marginal zone like cells may be seen at the periphery of nodules. Residual "naked" germinal centers are found in 50% of cases (Fig. 2.17). Tumor cells show a similar monotonous morphology as in other locations. This pattern of infiltration makes the differential diagnosis of MCL and other small cell lymphomas in the spleen difficult [142, 143].

Gastrointestinal Tract

A common manifestation of gastrointestinal disease is lymphomatoid polyposis, in which multiple lymphoid polyps are identified in the small and large bowel. These diffuse nodules may be associated with large tumor masses, usually ileocecal and

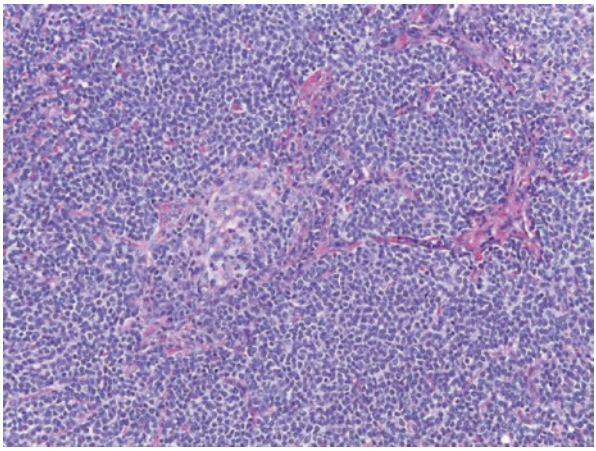

Fig. 2.17 Naked germinal center and prominent vessels

regional lymphadenopathy. Although this clinicopathologic presentation is relatively characteristic of MCL, differential diagnosis should include other non-Hodgkin's lymphomas, particularly follicular lymphoma and marginal zone lymphoma of the mucosa-associated lymphoid tissue (MALT) type. Cyclin D1 expression and BCL1 rearrangement are useful for the differential diagnosis of these tumors. MCL may also present as superficial ulcers, large tumor masses, and diffuse thickening of the mucosa. Microscopic infiltration of gastrointestinal mucosa by MCL without gross lesions is very common. In some cases glandular infiltration by tumor cells may mimic lymphoepithelial lesions, making the distinction between MCL and marginal zone lymphomas difficult [144].

Immunophenotype

The mantle cell lymphoma cells express B surface light chains (lambda more frequently than kappa) with moderate intensity and heavy chains (IgM/IgD). They are usually CD5, FMC-7, and CD43 positive and sometimes may express IFR4/MUM1. MCL cells express BCL2. They express B-cell markers CD19 and CD20 with brighter expression of CD20. Typically, CD23, CD10, CD11c, and CD25 expression is absent. Aberrant phenotypes are currently recognized including absence of CD5 expression and variable degree of CD10, BCL6, and CD200 (typically CLL marker) expression. The vast majority of cases demonstrates nuclear expression of cyclin D1 (>95% of cases) and SOX11 (>90% of cases) with immunohistochemical staining techniques. These stains are routinely applied in clinical practice in workup of lymph node biopsies. SOX11 immunostaining aids significantly in diagnosis of MCL as cyclin D1-negative and blastoid cases also demonstrate SOX11 positivity [3, 5, 21, 145–152].

CD5 expression is an important and helpful marker, which dictates consideration of MCL in the differential diagnosis of small-intermediate size neoplastic B-cell proliferation. The CD5 antigen is expressed by most T cells and a subset of B cells. In mice, CD5-positive B cells appear to be an independent B-cell lineage capable of self-renewal. CD5-positive B cells constitute a large proportion of the B cells in human fetal lymphoid tissue, although the percentage decreases with fetal age. In postnatal human lymphoid tissues, CD5-positive B cells are thought to be located predominantly in the mantle zone. The conventional (most common) form of MCL derives from mature B cells that have bypassed the germinal center and carry no or a limited number of IGHV mutations [34]. The second, less common, subtype also derives from cells that have the t(11;14) but have experienced the germinal center and carry a higher number of IGHV mutations. These two subsets retain DNA methylation and expression signatures reminiscent of naive and memory B cells, respectively [2, 16, 33, 36, 37].

Histologic Progression

Studies of sequential biopsies have shown that the histologic pattern of MCL usually remains relatively stable. Although progression from nodular to more diffuse pattern, changing patterns during evolution of disease, subsequent increase in large blastic cells, or mitotic activity were described, most blastoid MCLs are detected at diagnosis, and cytologic progression from classic MCL to a pure blastoid variant is relatively uncommon. When occasional progression from classic/conventional MCL to blastoid MCL was described, clonal relationship has been demonstrated. Tumor progression may manifest with the development of an overt leukemic phase [2, 6, 12, 15, 16, 127, 153].

Cytogenetics Abnormalities and Oncogenes

The characteristic cytogenetic alteration in MCL is t(11;14)(q13;q32), although variant translocations involving the 11q13 breakpoint have been reported [154]. This translocation is detected by conventional cytogenetics in up to 65% of MCLs and in virtually all cases by fluorescence in situ hybridization (FISH) [43, 155, 156]. However, t(11;14) translocations have also been identified in 5% of multiple myelomas [157]. Molecular analysis of this translocation in MCL and multiple myeloma suggests that the mechanism in these tumors may be different, with an error in the VDJ recombination in MCL and in the switch recombination process in myeloma. In addition, cyclin D1 gene amplification without translocation has been seen in cases of multiple myeloma but not in MCL [16, 158, 159].

Cytogenetic studies are employing a variety of methods such as conventional karyotype analysis, FISH, comparative genomic hybridization, and array-based analysis.[3] Array CGH (where the DNAs are directly labeled with Cy3 and Cy5 fluorescent dyes, with tumor DNA pseudocolored red and reference DNA green) and chromosomal CGH (where the differences in copy number between the tumor and normal DNA are reflected by differences in green and red fluorescence along the length of the chromosome [160]) were employed to characterize MCL cells and demonstrated a high number of secondary chromosomal alterations in MCL [16, 35–37, 69].

While recurrent translocations are relatively infrequent, with the exception of MYC and the t(11;14) hallmark, recurrent copy number alterations are common, with 90% of cases harboring at least one. Among commonly described are loss of 9p21/CDKN2A, 11q22/ATM, 13q14/Rb1, or 17p13/TP53 and gain/amplification of 8q24/MYC, 10p12/BMI1, 12q13/CDK4, or 18q21/BCL2, which affect genes involved in cell cycle regulation, DNA damage response, and cell survival (Table 2.4). Other recurrent alterations affect larger regions with no clear target genes, such as loss of 1p, 8p, or9q and gain of 3q, 7p, or 15q. Losses of chromosome 8p have been associated with a leukemic presentation in some studies but not in others. Blastoid variants have more complex karyotypes and more high-level DNA amplifications than classic variants. Gains of 3q, 7p, and 12q and losses of 17p are significantly more frequent in blastoid than classic variants. Tetraploidy is also more frequently associated with aggressive variants. Chromosome 8q24 alterations, including t(8;14)(q24;q32) and variants, seen in Burkitt's lymphoma, have been identified in occasional blastoid MCLs with a very aggressive clinical course. In addition to losses and gains of chromosomes/parts of chromosomes that lead to chromosomal imbalances that can be detected by conventional cytogenetics, mechanism of partial uniparental disomy (pUPD) was recently described in MCL. By loss of one or part of parental chromosome and gain of the homologue from the other parent, pUPD results in loss

Table 2.4 Chromosomal alterations in mantle cell lymphoma that contain altered genes shown to contribute to MCL pathogenesis

Chromosome region	Suggested target genes
Gains	
8q21-qter	MYC
11q13.3	*CCND1*
12q13	CDK4
18q11-q23	*BCL2*
Losses	
9p21-p22	*CDKN2A, ARF1*
17p13-ter	TP53
11q22-q23	ATM

Adapted from Ref. [16]

[3] Comparative genomic hybridization, CGH, is designed to scan the entire genome for unbalanced abnormalities – gains, losses, and amplification. In this method, tumor and reference (normal) DNAs are differentially labeled and cohybridized to normal metaphase spreads (chromosomal CGH) or to microarrays – thousands of DNA fragments of known sequenced arrayed in a known sequence on a chip (array CGHF).

of heterozygosity (LOH) without chromosomal deletion. As the gene dosage is not changed, pUPD cannot be detected by conventional cytogenetics, FISH or aCGH. Single-nucleotide polymorphisms (SNPs) assays were utilized to describe this mechanism. pUPD is considered an alternative mechanism for the inactivation of mutated tumor suppressor genes such as *p53* at 17p21 in MCL. All of these genetic alterations tend to accumulate in tumors with high proliferation and poor outcome [2, 16, 29, 33, 36, 37, 69, 70, 92, 161–164].

Prognosis and Predictive Factors

Prognosis is closely linked to the extent of disease at presentation. These factors are included into Mantle Cell Lymphoma International Prognostic Index (MIPI). MIPI is a risk score/formula calculated based on age, ECOG (Eastern Cooperative Oncology Group) performance status, serum LDH (lactate dehydrogenase) levels, and white cell count. MIPI less than 5.70 indicates low risk, MIPI of 5.70–6.2 is considered intermediate risk, and MIPI greater than 6.2 is considered high risk. About half of MCL patients have a high-risk MIPI score at presentation [2, 16, 165].

It was shown in many studies that *proliferative activity* is the most important prognostic parameter in MCL, independent of the method used for its evaluation. Thus, early studies showed that an increased mitotic index was an important prognostic indicator. The exact number of mitoses deemed significant varies, but a mitotic rate greater than 1.5–2.5 mitoses per high-power field generally indicates a more aggressive course. A high proliferative index recognized by Ki-67/MIB-1 immunostaining has also been associated with a poor prognosis, even in patients treated with rituximab [6, 12, 13, 65, 166, 167]. Although high proliferation is associated with blastoid morphology, tumors with classic cytology may also have high proliferative activity and an aggressive clinical course. Microarray analysis of the global expression profile in MCL has confirmed that quantification of the proliferation signature is the best predictor, defining subgroups of patients with more than 5 years median survival [65].

Ki-67 protein, a cellular marker for proliferation, is another important prognostic factor. A European Mantle Cell Lymphoma Network study showed that median overall survival for patients with a Ki-67 proliferation index of less than 30% was not reached and 5-year survival was 75%. At the same time, the median overall survival (OS) for those with Ki-67 proliferation index of 30% or greater was just 3.4 years, and 5-year OS was only 41% [165]. In limited series, a mantle zone *architectural growth pattern* has been associated with more frequent localized disease, a high proportion of complete remissions, and longer survival [12, 13, 16, 153]. The blastoid and pleomorphic *cytologic variants* are associated with a poor prognosis and a poor survival, with a median survival of approximately 14–18 months compared to 50 months for patients with a classic morphology. Blastoid variants are also associated with other negative prognostic markers such as high proliferative activity, increased cytogenetic alterations, and molecular alterations in tumor suppressor genes [12, 13, 16, 71]. Several studies have shown that *complex karyotype* is associated with poorer outcomes. *TP53 deletions* (which affect about 20% of MCL patients)

and mutations (which affect about 10%) are also useful prognostic factors, as each is associated with worse outcomes. A recent study showed that high TP53 staining (greater than 50% positive) is also associated with inferior outcomes, including reduced time to treatment failure and lower overall survival [2, 33, 71, 168–170].

Differential Diagnosis

Benign Disorders

Benign lymphoid hyperplasia may mimic MCL. Expanded primary lymphoid follicles and mantle zone hyperplasia seen in reactive lymph nodes or associated with Castleman's disease may suggest MCL with nodular or mantle zone patterns, respectively. The helpful features to distinguish these reactive conditions are the lack of nuclear irregularities observed in MCL, preservation of the nodal architecture, and clinical picture of localized lymphadenopathy in a young patient. CD5 and cyclin D1 negativity and lack of monoclonality help rule out the diagnosis of MCL [16, 134, 171].

Cyclin D1-Negative Mantle Cell Lymphoma

Small B-cell lymphoma may morphologically present with irregular nuclei resembling MCL but with negative cyclin D1. These cases also share 90% of the gene expression profile with cyclin D1-positive cases. In this situation, the differential diagnosis is difficult and may include three situations: false cyclin D1 negativity is due to technical failure, true cyclin D1-negative MCL, and other small B-cell lymphomas morphologically and phenotypically mimicking MCL. To rule out the first situation, it is important to investigate the presence of t(11;14) by FISH or assess the cyclin D1 expression by other methods such as quantitative PCR. The recognition of the cyclin D1-negative MCL is difficult because morphologically and phenotypically, similar lymphomas are also negative for cyclin D1 and t(11;14). These cyclin D1-negative B-cell lymphomas may even be $CD5^+$ and $CD23^-$. It was shown that cyclin D1-negative B-cell lymphomas are seen in younger patients, with less frequent gastrointestinal tract involved and better overall survival than in patients with MCL with cyclin D1 overexpression. About half of these cases show cyclin D2 rearrangements by FISH, and some cases show cyclin D2 overexpression by PCR. Some authors suggest that some cases of cyclin D1-negative MCL may represent different entities, including atypical B-cell CLL, $CD5^+$ marginal zone lymphoma, or lymphoplasmacytoid lymphoma. Given the important clinical impact of the diagnosis of MCL, experts caution about assigning definitive diagnosis in these cases. Implementation of SOX11 into clinical practice might help to better distinguish cases of cyclin D1-negative MCL as it is a relatively specific marker of MCL that is also consistently expressed in cyclin D1-negative MCL [16, 59, 64].

Disorders with t(11;14) Translocation

While t(11;14) is the hallmark translocation in MCL, t(11;14) translocations have also been identified in other hematologic malignancies. It was described in 5% of multiple myelomas [157]. Molecular analysis of this translocation in MCL and multiple myeloma suggests that the mechanism in these tumors may be different, with an error in the VDJ recombination in MCL and in the switch recombination process in myeloma. In addition, cyclin D1 gene amplification without translocation has been seen in cases of multiple myeloma but not in MCL [16, 158, 159].

Atypical Lymphocytosis

Some cases of MCL may present with atypical lymphocytosis without the morphology (round cells) or phenotype ($CD5^-$, $CD23^+$) suggestive of MCL. Some of these patients may have splenomegaly without peripheral lymphadenopathy, and they may be difficult to diagnose without cytogenetic or molecular studies. Previously defined cases of B-prolymphocytic leukemia with the t(11;14) translocation are reclassified as MCL, particularly the pleomorphic variants [2, 16, 26, 172].

Chronic Lymphocytic Leukemia: Small Lymphocytic Lymphoma

Typical CLL/SLL and MCL have distinctive morphologic characteristics (Table 2.5). However, some CLLs may have a high number of lymphocytes with "cleaved," irregular nuclei, mimicking MCL. CLL/SLL in lymph nodes may present with a predominant interfollicular pattern surrounding reactive secondary follicles. The predominance of small cells with round nuclei and the presence of prolymphocytes and paraimmunoblasts with central nucleoli usually point toward diagnosis of CLL/SLL as these findings are not seen in classic MCL. The different phenotypic characteristics of these entities may be useful in the differential. However, some overlap may exist, particularly when using flow cytometry, because some atypical CLLs may express strong CD20 and surface immunoglobulins, and dim CD23 may be detected in MCL. Assessment for t(11;14) by FISH may be very helpful in cases with atypical/overlapping features [2, 6, 8, 15, 16, 148, 149].

Follicular Lymphoma

Morphologic differentiation of nodular MCL from follicular lymphoma may be frequently problematic. Some cases of MCL might show a prominent nodular pattern, suggestive of follicular lymphoma. The monotonous cell population, with a

Table 2.5 Differential diagnosis of mantle cell lymphoma

Differential diagnosis	Similarities with MCL	Distinguishing features
CLL/SLL	Proliferation of small cell, CD5 expression	Prolymphocytes and paraimmunoblasts in CLL/SLL CD23 and CD200, dim CD20, and dim surface Ig expression in CLL/SLL Bright Cd20, surface Ig, and cyclin D1, t(11;14) in MCL
Follicular lymphoma (FL)	Nodular growth pattern	CD10/BCL6 expression, t(14;18) in FL CD5/cyclin D1 expression, t(11;14) in MCL
Marginal zone lymphoma (MZL)	Prominent monocytoid features in marginal-like zone MCL Some MZL cases are CD5-positive	Mixture of monocytoid, plasmacytoid, and large cells in MZL CD5/cyclin D1 expression, t(11;14) in MCL
Diffuse large-cell lymphoma (DLBCL)	Cytologic atypia and cell size in pleomorphic variant MCL DLBCL may express CD5 and cyclin D1	CD5, SOX11, and cyclin D1 coexpression in MCL along with t(11;14)
B-lymphoblastic lymphoma (B-LBL)	Blast-like features and high mitotic rate in blastoid MCL	TdT (terminal deoxynucleotidyl transferase) expression in B-LBL Surface Ig, Cd5, and cyclin D1 expression along with t(11;14) in MCL

lack of centroblasts and slightly fewer nuclear irregularities, should raise the possibility of MCL. However, occasional centroblasts representing cells from residual germinal centers may render the morphologic diagnosis of MCL difficult. Immunohistochemical staining for CD5, cyclin D1, CD10, and BCL6 typically helps to clarify the case. The differential diagnosis between follicular lymphoma with a diffuse pattern and diffuse MCL may also be difficult on histology alone, but addition of immunohistochemical stains makes it easier to distinguish them. The diagnosis of diffuse follicular lymphoma requires the presence of a minor centroblasts population and a typical follicular center cell phenotype with expression of CD10, BCL2, and BCL6 [2, 6, 8, 16, 145, 152, 173].

Marginal Zone Lymphoma

Some cases of MCL may have tumor cells with relatively abundant pale cytoplasm, which, coupled with the presence of residual germinal centers, may suggest the diagnosis of marginal zone lymphoma. However, the areas of conventional MCL can still be seen in these cases, and the absence of a mantle cell zone surrounding reactive germinal centers provides the clue for diagnosis of MCL. The

immunophenotype and molecular characteristics of MCL, with CD5 and cyclin D1 expression and IGH/CCND1 rearrangement, should confirm the diagnosis of MCL [2, 6, 16, 135, 145].

Diffuse Large B-Cell Lymphoma

Pleomorphic MCL may morphologically resemble large B-cell lymphoma. The large size of the cells and the occasional presence of a nucleolus may suggest this diagnosis. The nuclear characteristics of pleomorphic MCL (irregular cleaved contours, finely dispersed chromatin, and relative dissociation between the large nucleus and small nucleolus) might suggest a mantle cell origin. CD5 and cyclin D1 detection confirms the diagnosis of MCL. It is important to remember that a subset of large B-cell lymphomas may express CD5 antigen. These cases of large B-cell lymphomas do not carry the t(11;14) translocation, are cyclin D1 negative, and associated with an aggressive clinical course with shorter survival times than $CD5^-$ diffuse large B-cell lymphomas [2, 16, 139, 174].

Acute Leukemias

Blastoid MCL may present as a leukemic disorder with a very aggressive clinical course, mimicking acute myeloid or lymphoblastic leukemia. Clinically, these cases may represent evolution of a preexisting nodal disease or represent the initial leukemic manifestation of disease. The cytologic features of blastoid mantle cells with a high nuclear-to-cytoplasmic ratio, rounded nuclei with very finely dispersed chromatin, and small or inconspicuous nucleoli may suggest blasts. These forms express the typical MCL phenotype with strong B-cell markers, surface immunoglobulins, cyclin D1, and CD5; CD34 and terminal deoxynucleotidyl transferase are negative, thus allowing the differentiation between MCL and acute leukemias. In the cases of TdT and CD34, negative cases suspicious for B-lymphoblastic leukemia/lymphoma surface immunoglobulin light chain expression should be carefully assessed. Clonal surface immunoglobulin light chain expression is characteristically absent in immature B-lymphoblastic leukemia cells, while MCL cells typically demonstrate restricted (clonal) light chain expression. Cytogenetics and molecular studies may demonstrate the t (11;14) translocation or IGH/CCND1 rearrangement [2, 16, 25, 28].

Summary

- Mantle cell lymphoma (MCL) is a mature CD5+ B-cell non-Hodgkin lymphoma (NHL), which accounts for approximately 7% of adult NHLs in the United States and Europe. Its behavior is more often that of an aggressive lymphoma. Recently

more indolent subtypes (leukemic non-nodal mantle cell lymphoma, in situ mantle cell neoplasia) are recognized.

- Most MCL cases are postulated to derive from naïve pre-germinal center B cells of the mantle zone, while a subset of MCL may originate from marginal zone or peripheral blood memory B cells.
- Immunophenotype of typical MCL is of mature B cells with CD5 expression and negative for CD23, BCL6, and CD10.
- The genetic hallmark event is translocation t(11;14) which activates expression of the *CCND1* gene and pushes cell cycle progression from G1 to S phase.
- MCL, including cyclin D1-negative cases, is frequently associated with altered expression of SOX11, a transcription factor that may contribute to a block in differentiation in MCL cells.
- Multiple cytogenetic alterations and genes mutations highlight the importance of a decreased response to DNA damage, enhanced cell survival (impaired apoptosis), and constitutive activation of oncogenic pathways (such as classical NF-kB, alternative NF-kB, PI3K/AKT/mTOR, NOTCH, JAK/STAT3, WNT pathways) in the pathogenesis of MCL.
- Cyclin D1 overexpression and the presence of the t(11;14) translocation are key elements in the diagnosis.

References

1. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD, Jaffe ES. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127:2375–90.
2. Swerdlow SH. WHO classification of tumours of haematopoietic and lymphoid tissues (revised 4th edition). Lyon: IARC; 2017.
3. Swerdlow SH, Williams ME. From centrocytic to mantle cell lymphoma: a clinicopathologic and molecular review of 3 decades. Hum Pathol. 2002;33:7–20.
4. Lennert K, Stein H, Kaiserling E. Cytological and functional criteria for the classification of malignant lymphomata. Br J Cancer Suppl. 1975;2:29–43.
5. Tolksdorf G, Stein H, Lennert K. Morphological and immunological definition of a malignant lymphoma derived from germinal-centre cells with cleaved nuclei (centrocytes). Br J Cancer. 1980;41:168–82.
6. Swerdlow SH, Habeshaw JA, Murray LJ, Dhaliwal HS, Lister TA, Stansfeld AG. Centrocytic lymphoma: a distinct clinicopathologic and immunologic entity. A multiparameter study of 18 cases at diagnosis and relapse. Am J Pathol. 1983;113:181–97.
7. Stansfeld AG, Diebold J, Noel H, Kapanci Y, Rilke F, Kelenyi G, Sundstrom C, Lennert K, van Unnik JA, Mioduszewska O, et al. Updated Kiel classification for lymphomas. Lancet. 1988;1:292–3.
8. Swerdlow SH, Saboorian MH, Pelstring RJ, Williams ME. Centrocytic lymphoma: a morphometric study with comparison to other small cleaved follicular center cell lymphomas and genotypic correlates. Am J Pathol. 1993;142:329–37.
9. Banks PM, Chan J, Cleary ML, Delsol G, De Wolf-Peeters C, Gatter K, Grogan TM, Harris NL, Isaacson PG, Jaffe ES, et al. Mantle cell lymphoma. A proposal for unification of morphologic, immunologic, and molecular data. Am J Surg Pathol. 1992;16:637–40.
10. Zucca E, Stein H, Coiffier B. European lymphoma task force (ELTF): report of the workshop on mantle cell lymphoma (MCL). Ann Oncol. 1994;5:507–11.

11. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The non-Hodgkin's lymphoma classification project. Blood 1997;89:3909–18.
12. Argatoff LH, Connors JM, Klasa RJ, Horsman DE, Gascoyne RD. Mantle cell lymphoma: a clinicopathologic study of 80 cases. Blood. 1997;89:2067–78.
13. Bosch F, Lopez-Guillermo A, Campo E, Ribera JM, Conde E, Piris MA, Vallespi T, Woessner S, Montserrat E. Mantle cell lymphoma: presenting features, response to therapy, and prognostic factors. Cancer. 1998;82:567–75.
14. Campo E, Raffeld M, Jaffe ES. Mantle-cell lymphoma. Semin Hematol. 1999;36:115–27.
15. Lardelli P, Bookman MA, Sundeen J, Longo DL, Jaffe ES. Lymphocytic lymphoma of intermediate differentiation. Morphologic and immunophenotypic spectrum and clinical correlations. Am J Surg Pathol. 1990;14:752–63.
16. Jaffe ES. Hematopathology. Philadelphia, PA: Saunders/Elsevier Philadelphia, PA; 2011.
17. O'Briain DS, Kennedy MJ, Daly PA, O'Brien AA, Tanner WA, Rogers P, Lawlor E. Multiple lymphomatous polyposis of the gastrointestinal tract. A clinicopathologically distinctive form of non-Hodgkin's lymphoma of B-cell centrocytic type. Am J Surg Pathol. 1989;13:691–9.
18. Romaguera JE, Medeiros LJ, Hagemeister FB, Fayad LE, Rodriguez MA, Pro B, Younes A, McLaughlin P, Goy A, Sarris AH, Dang NH, Samaniego F, Brown HM, Gagneja HK, Cabanillas F. Frequency of gastrointestinal involvement and its clinical significance in mantle cell lymphoma. Cancer. 2003;97:586–91.
19. Montserrat E, Bosch F, Lopez-Guillermo A, Graus F, Terol MJ, Campo E, Rozman C. CNS involvement in mantle-cell lymphoma. J Clin Oncol Off J Am Soc Clin Oncol. 1996;14:941–4.
20. Oinonen R, Franssila K, Elonen E. Central nervous system involvement in patients with mantle cell lymphoma. Ann Hematol. 1999;78:145–9.
21. Samaha H, Dumontet C, Ketterer N, Moullet I, Thieblemont C, Bouafia F, Callet-Bauchu E, Felman P, Berger F, Salles G, Coiffier B. Mantle cell lymphoma: a retrospective study of 121 cases. Leukemia. 1998;12:1281–7.
22. Preudhomme JL. Loss of surface IgD by human B lymphocytes during polyclonal activation. Eur J Immunol. 1977;7:191–3.
23. Nanba K, Jaffe ES, Braylan RC, Soban EJ, Berard CW. Alkaline phosphatase-positive malignant lymphoma. A subtype of B-cell lymphomas. Am J Clin Pathol. 1977;68:535–42.
24. Pittaluga S, Verhoef G, Criel A, Maes A, Nuyts J, Boogaerts M, De Wolf PC. Prognostic significance of bone marrow trephine and peripheral blood smears in 55 patients with mantle cell lymphoma. Leuk Lymphoma. 1996;21:115–25.
25. Ferrer A, Salaverria I, Bosch F, Villamor N, Rozman M, Bea S, Gine E, Lopez-Guillermo A, Campo E, Montserrat E. Leukemic involvement is a common feature in mantle cell lymphoma. Cancer. 2007;109:2473–80.
26. Orchard J, Garand R, Davis Z, Babbage G, Sahota S, Matutes E, Catovsky D, Thomas PW, Avet-Loiseau H, Oscier D. A subset of t(11;14) lymphoma with mantle cell features displays mutated IgVH genes and includes patients with good prognosis, nonnodal disease. Blood. 2003;101:4975–81.
27. Vadlamudi G, Lionetti KA, Greenberg S, Mehta K. Leukemic phase of mantle cell lymphoma two case reports and review of the literature. Arch Pathol Lab Med. 1996;120:35–40.
28. Singleton TP, Anderson MM, Ross CW, Schnitzer B. Leukemic phase of mantle cell lymphoma, blastoid variant. Am J Clin Pathol. 1999;111:495–500.
29. Daniel MT, Tigaud I, Flexor MA, Nogueira ME, Berger R, Jonveaux P. Leukaemic non-Hodgkin's lymphomas with hyperdiploid cells and t(11;14)(q13;q32): a subtype of mantle cell lymphoma? Br J Haematol. 1995;90:77–84.
30. Vaishampayan UN, Mohamed AN, Dugan MC, Bloom RE, Palutke M. Blastic mantle cell lymphoma associated with Burkitt-type translocation and hypodiploidy. Br J Haematol. 2001;115:66–8.
31. Fernandez V, Salamero O, Espinet B, Sole F, Royo C, Navarro A, Camacho F, Bea S, Hartmann E, Amador V, Hernandez L, Agostinelli C, Sargent RL, Rozman M, Aymerich M, Colomer

D, Villamor N, Swerdlow SH, Pileri SA, Bosch F, Piris MA, Montserrat E, Ott G, Rosenwald A, Lopez-Guillermo A, Jares P, Serrano S, Campo E. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. Cancer Res. 2010;70:1408–18.
32. Royo C, Navarro A, Clot G, Salaverria I, Gine E, Jares P, Colomer D, Wiestner A, Wilson WH, Vegliante MC, Fernandez V, Hartmann EM, Trim N, Erber WN, Swerdlow SH, Klapper W, Dyer MJ, Vargas-Pabon M, Ott G, Rosenwald A, Siebert R, Lopez-Guillermo A, Campo E, Bea S. Non-nodal type of mantle cell lymphoma is a specific biological and clinical subgroup of the disease. Leukemia. 2012;26:1895–8.
33. Puente XS, Jares P, Campo E. Chronic lymphocytic leukemia and mantle cell lymphoma: crossroads of genetic and microenvironment interactions. Blood. 2018;131:2283–96.
34. Su W, Yeong KF, Spencer J. Immunohistochemical analysis of human CD5 positive B cells: mantle cells and mantle cell lymphoma are not equivalent in terms of CD5 expression. J Clin Pathol. 2000;53:395–7.
35. Jares P, Colomer D, Campo E. Molecular pathogenesis of mantle cell lymphoma. J Clin Invest. 2012;122:3416–23.
36. Vogt N, Dai B, Erdmann T, Berdel WE, Lenz G. The molecular pathogenesis of mantle cell lymphoma. Leuk Lymphoma. 2017;58:1530–7.
37. Perez-Galan P, Dreyling M, Wiestner A. Mantle cell lymphoma: biology, pathogenesis, and the molecular basis of treatment in the genomic era. Blood. 2011;117:26–38.
38. Jares P, Campo E. Advances in the understanding of mantle cell lymphoma. Br J Haematol. 2008;142:149–65.
39. Camacho FI, Algara P, Rodriguez A, Ruiz-Ballesteros E, Mollejo M, Martinez N, Martinez-Climent JA, Gonzalez M, Mateo M, Caleo A, Sanchez-Beato M, Menarguez J, Garcia-Conde J, Sole F, Campo E, Piris MA. Molecular heterogeneity in MCL defined by the use of specific VH genes and the frequency of somatic mutations. Blood. 2003;101:4042–6.
40. Kienle D, Krober A, Katzenberger T, Ott G, Leupolt E, Barth TF, Moller P, Benner A, Habermann A, Muller-Hermelink HK, Bentz M, Lichter P, Dohner H, Stilgenbauer S. VH mutation status and VDJ rearrangement structure in mantle cell lymphoma: correlation with genomic aberrations, clinical characteristics, and outcome. Blood. 2003;102:3003–9.
41. Li JY, Gaillard F, Moreau A, Harousseau JL, Laboisse C, Milpied N, Bataille R, Avet-Loiseau H. Detection of translocation t(11;14)(q13;q32) in mantle cell lymphoma by fluorescence in situ hybridization. Am J Pathol. 1999;154:1449–52.
42. Rosenberg CL, Wong E, Petty EM, Bale AE, Tsujimoto Y, Harris NL, Arnold A. PRAD1, a candidate BCL1 oncogene: mapping and expression in centrocytic lymphoma. Proc Natl Acad Sci U S A. 1991;88:9638–42.
43. Vaandrager JW, Schuuring E, Zwikstra E, de Boer CJ, Kleiverda KK, van Krieken JH, Kluin-Nelemans HC, van Ommen GJ, Raap AK, Kluin PM. Direct visualization of dispersed 11q13 chromosomal translocations in mantle cell lymphoma by multicolor DNA fiber fluorescence in situ hybridization. Blood. 1996;88:1177–82.
44. Vandenberghe E, De Wolf-Peeters C, van den Oord J, Wlodarska I, Delabie J, Stul M, Thomas J, Michaux JL, Mecucci C, Cassiman JJ, et al. Translocation (11;14): a cytogenetic anomaly associated with B-cell lymphomas of non-follicle centre cell lineage. J Pathol. 1991;163:13–8.
45. Williams ME, Swerdlow SH, Rosenberg CL, Arnold A. Characterization of chromosome 11 translocation breakpoints at the bcl-1 and PRAD1 loci in centrocytic lymphoma. Cancer research. 1992;52:5541s–4s.
46. Williams ME, Swerdlow SH, Meeker TC. Chromosome t(11;14)(q13;q32) breakpoints in centrocytic lymphoma are highly localized at the bcl-1 major translocation cluster. Leukemia. 1993;7:1437–40.
47. Williams ME, Swerdlow SH. Cyclin D1 overexpression in non-Hodgkin's lymphoma with chromosome 11 bcl-1 rearrangement. Ann Oncol. 1994;5 Suppl 1:71–3.
48. Arnold A, Kim HG, Gaz RD, Eddy RL, Fukushima Y, Byers MG, Shows TB, Kronenberg HM. Molecular cloning and chromosomal mapping of DNA rearranged with the parathyroid hormone gene in a parathyroid adenoma. J Clin Invest. 1989;83:2034–40.

49. Motokura T, Bloom T, Kim HG, Juppner H, Ruderman JV, Kronenberg HM, Arnold A. A novel cyclin encoded by a bcl1-linked candidate oncogene. Nature. 1991;350:512–5.
50. Hinds PW, Dowdy SF, Eaton EN, Arnold A, Weinberg RA. Function of a human cyclin gene as an oncogene. Proc Natl Acad Sci U S A. 1994;91:709–13.
51. Ewen ME, Sluss HK, Sherr CJ, Matsushime H, Kato J, Livingston DM. Functional interactions of the retinoblastoma protein with mammalian D-type cyclins. Cell. 1993;73:487–97.
52. Zukerberg LR, Benedict WF, Arnold A, Dyson N, Harlow E, Harris NL. Expression of the retinoblastoma protein in low-grade B-cell lymphoma: relationship to cyclin D1. Blood. 1996;88:268–76.
53. Lai R, Medeiros LJ, Coupland R, McCourty A, Brynes RK. Immunohistochemical detection of E2F-1 in non-Hodgkin's lymphomas: a survey of 124 cases. Mod Pathol. 1998;11:457–63.
54. Quintanilla-Martinez L, Thieblemont C, Fend F, Kumar S, Pinyol M, Campo E, Jaffe ES, Raffeld M. Mantle cell lymphomas lack expression of p27Kip1, a cyclin-dependent kinase inhibitor. Am J Pathol. 1998;153:175–82.
55. Quintanilla-Martinez L, Davies-Hill T, Fend F, Calzada-Wack J, Sorbara L, Campo E, Jaffe ES, Raffeld M. Sequestration of p27Kip1 protein by cyclin D1 in typical and blastic variants of mantle cell lymphoma (MCL): implications for pathogenesis. Blood. 2003;101:3181–7.
56. Lamb J, Ramaswamy S, Ford HL, Contreras B, Martinez RV, Kittrell FS, Zahnow CA, Patterson N, Golub TR, Ewen ME. A mechanism of cyclin D1 action encoded in the patterns of gene expression in human cancer. Cell. 2003;114:323–34.
57. Gan L, Liu P, Lu H, Chen S, Yang J, McCarthy JB, Knudsen KE, Huang H. Cyclin D1 promotes anchorage-independent cell survival by inhibiting FOXO-mediated anoikis. Cell Death Differ. 2009;16:1408–17.
58. Ek S, Dictor M, Jerkeman M, Jirstrom K, Borrebaeck CA. Nuclear expression of the non B-cell lineage Sox11 transcription factor identifies mantle cell lymphoma. Blood. 2008;111:800–5.
59. Mozos A, Royo C, Hartmann E, De Jong D, Baro C, Valera A, Fu K, Weisenburger DD, Delabie J, Chuang SS, Jaffe ES, Ruiz-Marcellan C, Dave S, Rimsza L, Braziel R, Gascoyne RD, Sole F, Lopez-Guillermo A, Colomer D, Staudt LM, Rosenwald A, Ott G, Jares P, Campo E. SOX11 expression is highly specific for mantle cell lymphoma and identifies the cyclin D1-negative subtype. Haematologica. 2009;94:1555–62.
60. Vegliante MC, Palomero J, Perez-Galan P, Roue G, Castellano G, Navarro A, Clot G, Moros A, Suarez-Cisneros H, Bea S, Hernandez L, Enjuanes A, Jares P, Villamor N, Colomer D, Martin-Subero JI, Campo E, Amador V. SOX11 regulates PAX5 expression and blocks terminal B-cell differentiation in aggressive mantle cell lymphoma. Blood. 2013;121:2175–85.
61. Palomero J, Vegliante MC, Eguileor A, Rodriguez ML, Balsas P, Martinez D, Campo E, Amador V. SOX11 defines two different subtypes of mantle cell lymphoma through transcriptional regulation of BCL6. Leukemia. 2016;30:1596–9.
62. Palomero J, Vegliante MC, Rodriguez ML, Eguileor A, Castellano G, Planas-Rigol E, Jares P, Ribera-Cortada I, Cid MC, Campo E, Amador V. SOX11 promotes tumor angiogenesis through transcriptional regulation of PDGFA in mantle cell lymphoma. Blood. 2014;124:2235–47.
63. Balsas P, Palomero J, Eguileor A, Rodriguez ML, Vegliante MC, Planas-Rigol E, Sureda-Gomez M, Cid MC, Campo E, Amador V. SOX11 promotes tumor protective microenvironment interactions through CXCR4 and FAK regulation in mantle cell lymphoma. Blood. 2017;130:501–13.
64. Yatabe Y, Suzuki R, Tobinai K, Matsuno Y, Ichinohasama R, Okamoto M, Yamaguchi M, Tamaru J, Uike N, Hashimoto Y, Morishima Y, Suchi T, Seto M, Nakamura S. Significance of cyclin D1 overexpression for the diagnosis of mantle cell lymphoma: a clinicopathologic comparison of cyclin D1-positive MCL and cyclin D1-negative MCL-like B-cell lymphoma. Blood. 2000;95:2253–61.
65. Rosenwald A, Wright G, Wiestner A, Chan WC, Connors JM, Campo E, Gascoyne RD, Grogan TM, Muller-Hermelink HK, Smeland EB, Chiorazzi M, Giltnane JM, Hurt EM, Zhao H, Averett L, Henrickson S, Yang L, Powell J, Wilson WH, Jaffe ES, Simon R, Klausner RD,

Montserrat E, Bosch F, Greiner TC, Weisenburger DD, Sanger WG, Dave BJ, Lynch JC, Vose J, Armitage JO, Fisher RI, Miller TP, LeBlanc M, Ott G, Kvaloy S, Holte H, Delabie J, Staudt LM. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. Cancer Cell. 2003;3:185–97.

66. Fu K, Weisenburger DD, Greiner TC, Dave S, Wright G, Rosenwald A, Chiorazzi M, Iqbal J, Gesk S, Siebert R, De Jong D, Jaffe ES, Wilson WH, Delabie J, Ott G, Dave BJ, Sanger WG, Smith LM, Rimsza L, Braziel RM, Muller-Hermelink HK, Campo E, Gascoyne RD, Staudt LM, Chan WC. Lymphoma/Leukemia Molecular Profiling P: Cyclin D1-negative mantle cell lymphoma: a clinicopathologic study based on gene expression profiling. Blood. 2005;106:4315–21.
67. Salaverria I, Royo C, Carvajal-Cuenca A, Clot G, Navarro A, Valera A, Song JY, Woroniecka R, Rymkiewicz G, Klapper W, Hartmann EM, Sujobert P, Wlodarska I, Ferry JA, Gaulard P, Ott G, Rosenwald A, Lopez-Guillermo A, Quintanilla-Martinez L, Harris NL, Jaffe ES, Siebert R, Campo E, Bea S. CCND2 rearrangements are the most frequent genetic events in cyclin D1(-) mantle cell lymphoma. Blood. 2013;121:1394–402.
68. Fernandez V, Hartmann E, Ott G, Campo E, Rosenwald A. Pathogenesis of mantle-cell lymphoma: all oncogenic roads lead to dysregulation of cell cycle and DNA damage response pathways. J Clin Oncol Off J Am Soc Clin Oncol. 2005;23:6364–9.
69. Jares P, Colomer D, Campo E. Genetic and molecular pathogenesis of mantle cell lymphoma: perspectives for new targeted therapeutics. Nat Rev Cancer. 2007;7:750–62.
70. Bea S, Valdes-Mas R, Navarro A, Salaverria I, Martin-Garcia D, Jares P, Gine E, Pinyol M, Royo C, Nadeu F, Conde L, Juan M, Clot G, Vizan P, Di Croce L, Puente DA, Lopez-Guerra M, Moros A, Roue G, Aymerich M, Villamor N, Colomo L, Martinez A, Valera A, Martin-Subero JI, Amador V, Hernandez L, Rozman M, Enjuanes A, Forcada P, Muntanola A, Hartmann EM, Calasanz MJ, Rosenwald A, Ott G, Hernandez-Rivas JM, Klapper W, Siebert R, Wiestner A, Wilson WH, Colomer D, Lopez-Guillermo A, Lopez-Otin C, Puente XS, Campo E. Landscape of somatic mutations and clonal evolution in mantle cell lymphoma. Proc Natl Acad Sci U S A. 2013;110:18250–5.
71. Hernandez L, Fest T, Cazorla M, Teruya-Feldstein J, Bosch F, Peinado MA, Piris MA, Montserrat E, Cardesa A, Jaffe ES, Campo E, Raffeld M. p53 gene mutations and protein overexpression are associated with aggressive variants of mantle cell lymphomas. Blood. 1996;87:3351–9.
72. Pant V, Lozano G. Limiting the power of p53 through the ubiquitin proteasome pathway. Genes Dev. 2014;28:1739–51.
73. Parrales A, Iwakuma T. Targeting oncogenic mutant p53 for cancer therapy. Front Oncol. 2015;5:288.
74. Muller PA, Vousden KH. Mutant p53 in cancer: new functions and therapeutic opportunities. Cancer Cell. 2014;25:304–17.
75. Weinberg RA. The biology of cancer (second edition). New York: Garland Science, Taylo & Francis Group, LLC; 2014.
76. Halldorsdottir AM, Lundin A, Murray F, Mansouri L, Knuutila S, Sundstrom C, Laurell A, Ehrencrona H, Sander B, Rosenquist R. Impact of TP53 mutation and 17p deletion in mantle cell lymphoma. Leukemia. 2011;25:1904–8.
77. Rivlin N, Koifman G, Rotter V. p53 orchestrates between normal differentiation and cancer. Semin Cancer Biol. 2015;32:10–7.
78. Joerger AC, Fersht AR. Structural biology of the tumor suppressor p53 and cancer-associated mutants. Adv Cancer Res. 2007;97:1–23.
79. Hernandez L, Bea S, Pinyol M, Ott G, Katzenberger T, Rosenwald A, Bosch F, Lopez-Guillermo A, Delabie J, Colomer D, Montserrat E, Campo E. CDK4 and MDM2 gene alterations mainly occur in highly proliferative and aggressive mantle cell lymphomas with wild-type INK4a/ARF locus. Cancer Res. 2005;65:2199–206.
80. Choi M, Kipps T, Kurzrock R. ATM mutations in cancer: therapeutic implications. Mol Cancer Ther. 2016;15:1781–91.

81. Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkenazi M, Pecker I, Frydman M, Harnik R, Patanjali SR, Simmons A, Clines GA, Sartiel A, Gatti RA, Chessa L, Sanal O, Lavin MF, Jaspers NG, Taylor AM, Arlett CF, Miki T, Weissman SM, Lovett M, Collins FS, Shiloh Y. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science. 1995;268:1749–53.
82. Bednarski JJ, Sleckman BP. Integrated signaling in developing lymphocytes: the role of DNA damage responses. Cell Cycle. 2012;11:4129–34.
83. Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu Rev Biochem. 2010;79:181–211.
84. Shiloh Y, Ziv Y. The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. Nat Rev Mol Cell Biol. 2013;14:197–210.
85. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature. 2003;421:499–506.
86. Lavin MF, Kozlov S. ATM activation and DNA damage response. Cell Cycle. 2007;6:931–42.
87. Mochan TA, Venere M, DiTullio RA Jr, Halazonetis TD. 53BP1 and NFBD1/MDC1-Nbs1 function in parallel interacting pathways activating ataxia-telangiectasia mutated (ATM) in response to DNA damage. Cancer Res. 2003;63:8586–91.
88. Blasius M, Bartek J. ATM targets hnRNPK to control p53. Cell Cycle. 2013;12:1162–3.
89. Camacho E, Hernandez L, Hernandez S, Tort F, Bellosillo B, Bea S, Bosch F, Montserrat E, Cardesa A, Fernandez PL, Campo E. ATM gene inactivation in mantle cell lymphoma mainly occurs by truncating mutations and missense mutations involving the phosphatidylinositol-3 kinase domain and is associated with increasing numbers of chromosomal imbalances. Blood. 2002;99:238–44.
90. Dombernowsky SL, Weischer M, Allin KH, Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG. Risk of cancer by ATM missense mutations in the general population. J Clin Oncol Off J Am Soc Clin Oncol. 2008;26:3057–62.
91. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene. 2007;26:1324–37.
92. Bea S, Salaverria I, Armengol L, Pinyol M, Fernandez V, Hartmann EM, Jares P, Amador V, Hernandez L, Navarro A, Ott G, Rosenwald A, Estivill X, Campo E. Uniparental disomies, homozygous deletions, amplifications, and target genes in mantle cell lymphoma revealed by integrative high-resolution whole-genome profiling. Blood. 2009;113:3059–69.
93. Wenzel SS, Grau M, Mavis C, Hailfinger S, Wolf A, Madle H, Deeb G, Dorken B, Thome M, Lenz P, Dirnhofer S, Hernandez-Ilizaliturri FJ, Tzankov A, Lenz G. MCL1 is deregulated in subgroups of diffuse large B-cell lymphoma. Leukemia. 2013;27:1381–90.
94. Shishodia S, Amin HM, Lai R, Aggarwal BB. Curcumin (diferuloylmethane) inhibits constitutive NF-kappaB activation, induces G1/S arrest, suppresses proliferation, and induces apoptosis in mantle cell lymphoma. Biochem Pharmacol. 2005;70:700–13.
95. Rizzatti EG, Falcao RP, Panepucci RA, Proto-Siqueira R, Anselmo-Lima WT, Okamoto OK, Zago MA. Gene expression profiling of mantle cell lymphoma cells reveals aberrant expression of genes from the PI3K-AKT, WNT and TGFbeta signalling pathways. Br J Haematol. 2005;130:516–26.
96. Rudelius M, Pittaluga S, Nishizuka S, Pham TH, Fend F, Jaffe ES, Quintanilla-Martinez L, Raffeld M. Constitutive activation of Akt contributes to the pathogenesis and survival of mantle cell lymphoma. Blood. 2006;108:1668–76.
97. Psyrri A, Papageorgiou S, Liakata E, Scorilas A, Rontogianni D, Kontos CK, Argyriou P, Pectasides D, Harhalakis N, Pappa V, Kolialexi A, Economopoulou C, Kontsioti F, Maratou E, Dimitriadis G, Economopoulou P, Economopoulos T. Phosphatidylinositol 3′-kinase catalytic subunit alpha gene amplification contributes to the pathogenesis of mantle cell lymphoma. Clin Cancer Res. 2009;15:5724–32.
98. Dal Col J, Zancai P, Terrin L, Guidoboni M, Ponzoni M, Pavan A, Spina M, Bergamin S, Rizzo S, Tirelli U, De Rossi A, Doglioni C, Dolcetti R. Distinct functional significance of Akt and mTOR constitutive activation in mantle cell lymphoma. Blood. 2008;111:5142–51.

99. Karin M, Lin A. NF-kappaB at the crossroads of life and death. Nat Immunol. 2002;3:221–7.
100. Ghosh S, Hayden MS. Celebrating 25 years of NF-kappaB research. Immunol Rev. 2012;246:5–13.
101. Pham LV, Tamayo AT, Yoshimura LC, Lo P, Ford RJ. Inhibition of constitutive NF-kappa B activation in mantle cell lymphoma B cells leads to induction of cell cycle arrest and apoptosis. J Immunol. 2003;171:88–95.
102. Rahal R, Frick M, Romero R, Korn JM, Kridel R, Chan FC, Meissner B, Bhang HE, Ruddy D, Kauffmann A, Farsidjani A, Derti A, Rakiec D, Naylor T, Pfister E, Kovats S, Kim S, Dietze K, Dorken B, Steidl C, Tzankov A, Hummel M, Monahan J, Morrissey MP, Fritsch C, Sellers WR, Cooke VG, Gascoyne RD, Lenz G, Stegmeier F. Pharmacological and genomic profiling identifies NF-kappaB-targeted treatment strategies for mantle cell lymphoma. Nat Med. 2014;20:87–92.
103. Roue G, Perez-Galan P, Lopez-Guerra M, Villamor N, Campo E, Colomer D. Selective inhibition of IkappaB kinase sensitizes mantle cell lymphoma B cells to TRAIL by decreasing cellular FLIP level. J Immunol. 2007;178:1923–30.
104. Rinaldi A, Kwee I, Taborelli M, Largo C, Uccella S, Martin V, Poretti G, Gaidano G, Calabrese G, Martinelli G, Baldini L, Pruneri G, Capella C, Zucca E, Cotter FE, Cigudosa JC, Catapano CV, Tibiletti MG, Bertoni F. Genomic and expression profiling identifies the B-cell associated tyrosine kinase Syk as a possible therapeutic target in mantle cell lymphoma. Br J Haematol. 2006;132:303–16.
105. Fu L, Lin-Lee YC, Pham LV, Tamayo AT, Yoshimura LC, Ford RJ. BAFF-R promotes cell proliferation and survival through interaction with IKKbeta and NF-kappaB/c-Rel in the nucleus of normal and neoplastic B-lymphoid cells. Blood. 2009;113:4627–36.
106. Honma K, Tsuzuki S, Nakagawa M, Tagawa H, Nakamura S, Morishima Y, Seto M. TNFAIP3/A20 functions as a novel tumor suppressor gene in several subtypes of non-Hodgkin lymphomas. Blood. 2009;114:2467–75.
107. Tanigaki K, Kuroda K, Han H, Honjo T. Regulation of B cell development by Notch/RBP-J signaling. Semin Immunol. 2003;15:113–9.
108. Guruharsha KG, Kankel MW, Artavanis-Tsakonas S. The Notch signalling system: recent insights into the complexity of a conserved pathway. Nat Rev Genet. 2012;13:654–66.
109. Kridel R, Meissner B, Rogic S, Boyle M, Telenius A, Woolcock B, Gunawardana J, Jenkins C, Cochrane C, Ben-Neriah S, Tan K, Morin RD, Opat S, Sehn LH, Connors JM, Marra MA, Weng AP, Steidl C, Gascoyne RD. Whole transcriptome sequencing reveals recurrent NOTCH1 mutations in mantle cell lymphoma. Blood. 2012;119:1963–71.
110. Ryan RJH, Petrovic J, Rausch DM, Zhou Y, Lareau CA, Kluk MJ, Christie AL, Lee WY, Tarjan DR, Guo B, Donohue LKH, Gillespie SM, Nardi V, Hochberg EP, Blacklow SC, Weinstock DM, Faryabi RB, Bernstein BE, Aster JC, Pear WS. A B cell regulome links notch to downstream oncogenic pathways in small B cell lymphomas. Cell Rep. 2017;21:784–97.
111. Arruga F, Vaisitti T, Deaglio S. The NOTCH pathway and its mutations in mature B cell malignancies. Front Oncol. 2018;8:550.
112. Leshchenko VV, Kuo PY, Shaknovich R, Yang DT, Gellen T, Petrich A, Yu Y, Remache Y, Weniger MA, Rafiq S, Suh KS, Goy A, Wilson W, Verma A, Braunschweig I, Muthusamy N, Kahl BS, Byrd JC, Wiestner A, Melnick A, Parekh S. Genomewide DNA methylation analysis reveals novel targets for drug development in mantle cell lymphoma. Blood. 2010;116:1025–34.
113. Yu H, Lee H, Herrmann A, Buettner R, Jove R. Revisiting STAT3 signalling in cancer: new and unexpected biological functions. Nat Rev Cancer. 2014;14:736–46.
114. Baran-Marszak F, Boukhiar M, Harel S, Laguillier C, Roger C, Gressin R, Martin A, Fagard R, Varin-Blank N, Ajchenbaum-Cymbalista F, Ledoux D. Constitutive and B-cell receptor-induced activation of STAT3 are important signaling pathways targeted by bortezomib in leukemic mantle cell lymphoma. Haematologica. 2010;95:1865–72.
115. Lai R, Rassidakis GZ, Medeiros LJ, Leventaki V, Keating M, McDonnell TJ. Expression of STAT3 and its phosphorylated forms in mantle cell lymphoma cell lines and tumours. J Pathol. 2003;199:84–9.

116. Chim CS, Wong KY, Loong F, Srivastava G. SOCS1 and SHP1 hypermethylation in mantle cell lymphoma and follicular lymphoma: implications for epigenetic activation of the Jak/STAT pathway. Leukemia. 2004;18:356–8.
117. Gelebart P, Anand M, Armanious H, Peters AC, Dien Bard J, Amin HM, Lai R. Constitutive activation of the Wnt canonical pathway in mantle cell lymphoma. Blood. 2008;112:5171–9.
118. Anastas JN, Moon RT. WNT signalling pathways as therapeutic targets in cancer. Nat Rev Cancer. 2013;13:11–26.
119. Chung R, Peters AC, Armanious H, Anand M, Gelebart P, Lai R. Biological and clinical significance of GSK-3beta in mantle cell lymphoma – an immunohistochemical study. Int J Clin Exp Pathol. 2010;3:244–53.
120. Ortega-Paino E, Fransson J, Ek S, Borrebaeck CA. Functionally associated targets in mantle cell lymphoma as defined by DNA microarrays and RNA interference. Blood. 2008;111:1617–24.
121. Bernard S, Danglade D, Gardano L, Laguillier C, Lazarian G, Roger C, Thieblemont C, Marzec J, Gribben J, Cymbalista F, Varin-Blank N, Ledoux D, Baran-Marszak F. Inhibitors of BCR signalling interrupt the survival signal mediated by the micro-environment in mantle cell lymphoma. Int J Cancer. 2015;136:2761–74.
122. Dai B, Grau M, Juilland M, Klener P, Horing E, Molinsky J, Schimmack G, Aukema SM, Hoster E, Vogt N, Staiger AM, Erdmann T, Xu W, Erdmann K, Dzyuba N, Madle H, Berdel WE, Trneny M, Dreyling M, Johrens K, Lenz P, Rosenwald A, Siebert R, Tzankov A, Klapper W, Anagnostopoulos I, Krappmann D, Ott G, Thome M, Lenz G. B-cell receptor-driven MALT1 activity regulates MYC signaling in mantle cell lymphoma. Blood. 2017;129:333–46.
123. Saba NS, Liu D, Herman SE, Underbayev C, Tian X, Behrend D, Weniger MA, Skarzynski M, Gyamfi J, Fontan L, Melnick A, Grant C, Roschewski M, Navarro A, Bea S, Pittaluga S, Dunleavy K, Wilson WH, Wiestner A. Pathogenic role of B-cell receptor signaling and canonical NF-kappaB activation in mantle cell lymphoma. Blood. 2016;128:82–92.
124. Kurtova AV, Tamayo AT, Ford RJ, Burger JA. Mantle cell lymphoma cells express high levels of CXCR4, CXCR5, and VLA-4 (CD49d): importance for interactions with the stromal microenvironment and specific targeting. Blood. 2009;113:4604–13.
125. Corcione A, Arduino N, Ferretti E, Raffaghello L, Roncella S, Rossi D, Fedeli F, Ottonello L, Trentin L, Dallegri F, Semenzato G, Pistoia V. CCL19 and CXCL12 trigger in vitro chemotaxis of human mantle cell lymphoma B cells. Clin Cancer Res. 2004;10:964–71.
126. Hutter G, Scheubner M, Zimmermann Y, Kalla J, Katzenberger T, Hubler K, Roth S, Hiddemann W, Ott G, Dreyling M. Differential effect of epigenetic alterations and genomic deletions of CDK inhibitors [p16(INK4a), p15(INK4b), p14(ARF)] in mantle cell lymphoma. Genes Chromosomes Cancer. 2006;45:203–10.
127. Vogt N, Klapper W. Variability in morphology and cell proliferation in sequential biopsies of mantle cell lymphoma at diagnosis and relapse: clinical correlation and insights into disease progression. Histopathology. 2013;62:334–42.
128. Zhang J, Jima D, Moffitt AB, Liu Q, Czader M, Hsi ED, Fedoriw Y, Dunphy CH, Richards KL, Gill JI, Sun Z, Love C, Scotland P, Lock E, Levy S, Hsu DS, Dunson D, Dave SS. The genomic landscape of mantle cell lymphoma is related to the epigenetically determined chromatin state of normal B cells. Blood. 2014;123:2988–96.
129. Carvajal-Cuenca A, Sua LF, Silva NM, Pittaluga S, Royo C, Song JY, Sargent RL, Espinet B, Climent F, Jacobs SA, Delabie J, Naresh KN, Bagg A, Brousset P, Warnke RA, Serrano S, Harris NL, Swerdlow SH, Jaffe ES, Campo E. In situ mantle cell lymphoma: clinical implications of an incidental finding with indolent clinical behavior. Haematologica. 2012;97:270–8.
130. Karube K, Scarfo L, Campo E, Ghia P. Monoclonal B cell lymphocytosis and “in situ” lymphoma. Semin Cancer Biol. 2014;24:3–14.
131. Nodit L, Bahler DW, Jacobs SA, Locker J, Swerdlow SH. Indolent mantle cell lymphoma with nodal involvement and mutated immunoglobulin heavy chain genes. Hum Pathol. 2003;34:1030–4.
132. Richard P, Vassallo J, Valmary S, Missoury R, Delsol G, Brousset P. “In situ-like” mantle cell lymphoma: a report of two cases. J Clin Pathol. 2006;59:995–6.

133. Majlis A, Pugh WC, Rodriguez MA, Benedict WF, Cabanillas F. Mantle cell lymphoma: correlation of clinical outcome and biologic features with three histologic variants. J Clin Oncol Off J Am Soc Clin Oncol. 1997;15:1664–71.
134. Hunt JP, Chan JA, Samoszuk M, Brynes RK, Hernandez AM, Bass R, Weisenburger DD, Muller-Hermelink K, Nathwani BN. Hyperplasia of mantle/marginal zone B cells with clear cytoplasm in peripheral lymph nodes. A clinicopathologic study of 35 cases. Am J Clin Pathol. 2001;116:550–9.
135. Young KH, Chan WC, Fu K, Iqbal J, Sanger WG, Ratashak A, Greiner TC, Weisenburger DD. Mantle cell lymphoma with plasma cell differentiation. Am J Surg Pathol. 2006;30:954–61.
136. Swerdlow SH, Zukerberg LR, Yang WI, Harris NL, Williams ME. The morphologic spectrum of non-Hodgkin's lymphomas with BCL1/cyclin D1 gene rearrangements. Am J Surg Pathol. 1996;20:627–40.
137. Pinyol M, Cobo F, Bea S, Jares P, Nayach I, Fernandez PL, Montserrat E, Cardesa A, Campo E. p16(INK4a) gene inactivation by deletions, mutations, and hypermethylation is associated with transformed and aggressive variants of non-Hodgkin's lymphomas. Blood. 1998;91:2977–84.
138. Dreyling MH, Bullinger L, Ott G, Stilgenbauer S, Muller-Hermelink HK, Bentz M, Hiddemann W, Dohner H. Alterations of the cyclin D1/p16-pRB pathway in mantle cell lymphoma. Cancer Res. 1997;57:4608–14.
139. Ott MM, Ott G, Kuse R, Porowski P, Gunzer U, Feller AC, Muller-Hermelink HK. The anaplastic variant of centrocytic lymphoma is marked by frequent rearrangements of the bcl-1 gene and high proliferation indices. Histopathology. 1994;24:329–34.
140. Wasman J, Rosenthal NS, Farhi DC. Mantle cell lymphoma. Morphologic findings in bone marrow involvement. Am J Clin Pathol. 1996;106:196–200.
141. Vasef MA, Medeiros LJ, Koo C, McCourty A, Brynes RK. Cyclin D1 immunohistochemical staining is useful in distinguishing mantle cell lymphoma from other low-grade B-cell neoplasms in bone marrow. Am J Clin Pathol. 1997;108:302–7.
142. Arber DA, Rappaport H, Weiss LM. Non-Hodgkin's lymphoproliferative disorders involving the spleen. Mod Pathol. 1997;10:18–32.
143. Wong KF, So CC, Chan JK. Nucleolated variant of mantle cell lymphoma with leukemic manifestations mimicking prolymphocytic leukemia. Am J Clin Pathol. 2002;117:246–51.
144. Kumar S, Krenacs L, Otsuki T, Kumar D, Harris CA, Wellmann A, Jaffe ES. Raffeld M: bc1-1 rearrangement and cyclin D1 protein expression in multiple lymphomatous polyposis. Am J Clin Pathol. 1996;105:737–43.
145. Stein H, Staudinger M, Tolksdorf G, Lennert K. Immunologic markers in the differential diagnosis of non-Hodgkin's lymphomas. J Cancer Res Clin Oncol. 1981;101:29–42.
146. Swerdlow SH, Utz GL, Williams ME. Bcl-2 protein in centrocytic lymphoma; a paraffin section study. Leukemia. 1993;7:1456–8.
147. Swerdlow SH, Yang WI, Zukerberg LR, Harris NL, Arnold A, Williams ME. Expression of cyclin D1 protein in centrocytic/mantle cell lymphomas with and without rearrangement of the BCL1/cyclin D1 gene. Hum Pathol. 1995;26:999–1004.
148. Dorfman DM, Pinkus GS. Distinction between small lymphocytic and mantle cell lymphoma by immunoreactivity for CD23. Mod Pathol. 1994;7:326–31.
149. Kumar S, Green GA, Teruya-Feldstein J, Raffeld M, Jaffe ES. Use of CD23 (BU38) on paraffin sections in the diagnosis of small lymphocytic lymphoma and mantle cell lymphoma. Mod Pathol. 1996;9:925–9.
150. Gong JZ, Lagoo AS, Peters D, Horvatinovich J, Benz P, Buckley PJ. Value of CD23 determination by flow cytometry in differentiating mantle cell lymphoma from chronic lymphocytic leukemia/small lymphocytic lymphoma. Am J Clin Pathol. 2001;116:893–7.
151. Camacho FI, Garcia JF, Cigudosa JC, Mollejo M, Algara P, Ruiz-Ballesteros E, Gonzalvo P, Martin P, Perez-Seoane C, Sanchez-Garcia J, Piris MA. Aberrant Bcl6 protein expression in mantle cell lymphoma. Am J Surg Pathol. 2004;28:1051–6.

152. Zanetto U, Dong H, Huang Y, Zhang K, Narbaitz M, Sapia S, Kostopoulos I, Liu H, Du MQ, Bacon CM. Mantle cell lymphoma with aberrant expression of CD10. Histopathology. 2008;53:20–9.
153. Norton AJ, Matthews J, Pappa V, Shamash J, Love S, Rohatiner AZ, Lister TA. Mantle cell lymphoma: natural history defined in a serially biopsied population over a 20-year period. Ann Oncol. 1995;6:249–56.
154. Komatsu H, Iida S, Yamamoto K, Mikuni C, Nitta M, Takahashi T, Ueda R, Seto M. A variant chromosome translocation at 11q13 identifying PRAD1/cyclin D1 as the BCL-1 gene. Blood. 1994;84:1226–31.
155. Bigoni R, Negrini M, Veronese ML, Cuneo A, Castoldi GL, Croce CM. Characterization of t(11;14) translocation in mantle cell lymphoma by fluorescent in situ hybridization. Oncogene. 1996;13:797–802.
156. Monteil M, Callanan M, Dascalescu C, Sotto JJ, Leroux D. Molecular diagnosis of t(11;14) in mantle cell lymphoma using two-colour interphase fluorescence in situ hybridization. Br J Haematol. 1996;93:656–60.
157. Raffeld M. Jaffe ES: bcl-1, t(11;14), and mantle cell-derived lymphomas. Blood. 1991;78:259–63.
158. Hoechtlen-Vollmar W, Menzel G, Bartl R, Lamerz R, Wick M, Seidel D. Amplification of cyclin D1 gene in multiple myeloma: clinical and prognostic relevance. Br J Haematol. 2000;109:30–8.
159. Chesi M, Bergsagel PL, Brents LA, Smith CM, Gerhard DS, Kuehl WM. Dysregulation of cyclin D1 by translocation into an IgH gamma switch region in two multiple myeloma cell lines. Blood. 1996;88:674–81.
160. Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science. 1992;258:818–21.
161. Bentz M, Plesch A, Bullinger L, Stilgenbauer S, Ott G, Muller-Hermelink HK, Baudis M, Barth TF, Moller P, Lichter P, Dohner H. t(11;14)-positive mantle cell lymphomas exhibit complex karyotypes and share similarities with B-cell chronic lymphocytic leukemia. Genes Chromosomes Cancer. 2000;27:285–94.
162. Martinez-Climent JA, Vizcarra E, Sanchez D, Blesa D, Marugan I, Benet I, Sole F, Rubio-Moscardo F, Terol MJ, Climent J, Sarsotti E, Tormo M, Andreu E, Salido M, Ruiz MA, Prosper F, Siebert R, Dyer MJ, Garcia-Conde J. Loss of a novel tumor suppressor gene locus at chromosome 8p is associated with leukemic mantle cell lymphoma. Blood. 2001;98:3479–82.
163. Levy V, Ugo V, Delmer A, Tang R, Ramond S, Perrot JY, Vrhovac R, Marie JP, Zittoun R, Ajchenbaum-Cymbalista F. Cyclin D1 overexpression allows identification of an aggressive subset of leukemic lymphoproliferative disorder. Leukemia. 1999;13:1343–51.
164. Onciu M, Schlette E, Medeiros LJ, Abruzzo LV, Keating M, Lai R. Cytogenetic findings in mantle cell lymphoma cases with a high level of peripheral blood involvement have a distinct pattern of abnormalities. Am J Clin Pathol. 2001;116:886–92.
165. Hoster E, Rosenwald A, Berger F, Bernd HW, Hartmann S, Loddenkemper C, Barth TF, Brousse N, Pileri S, Rymkiewicz G, Kodet R, Stilgenbauer S, Forstpointner R, Thieblemont C, Hallek M, Coiffier B, Vehling-Kaiser U, Bouabdallah R, Kanz L, Pfreundschuh M, Schmidt C, Ribrag V, Hiddemann W, Unterhalt M, Kluin-Nelemans JC, Hermine O, Dreyling MH, Klapper W. Prognostic value of Ki-67 index, cytology, and growth pattern in mantle-cell lymphoma: results from randomized trials of the European Mantle Cell Lymphoma Network. J Clin Oncol. 2016;34:1386–94.
166. Salaverria I, Zettl A, Bea S, Moreno V, Valls J, Hartmann E, Ott G, Wright G, Lopez-Guillermo A, Chan WC, Weisenburger DD, Gascoyne RD, Grogan TM, Delabie J, Jaffe ES, Montserrat E, Muller-Hermelink HK, Staudt LM, Rosenwald A, Campo E. Specific secondary genetic alterations in mantle cell lymphoma provide prognostic information independent of the gene expression-based proliferation signature. J Clin Oncol. 2007;25:1216–22.
167. Determann O, Hoster E, Ott G, Wolfram Bernd H, Loddenkemper C, Leo Hansmann M, Barth TE, Unterhalt M, Hiddemann W, Dreyling M, Klapper W, European Mantle Cell Lymphoma N, the German Low Grade Lymphoma Study G. Ki-67 predicts outcome in advanced-stage

mantle cell lymphoma patients treated with anti-CD20 immunochemotherapy: results from randomized trials of the European MCL Network and the German Low Grade Lymphoma Study Group. Blood. 2008;111:2385–7.
168. Greenwell IB, Staton AD, Lee MJ, Switchenko JM, Saxe DF, Maly JJ, Blum KA, Grover NS, Mathews SP, Gordon MJ, Danilov AV, Epperla N, Fenske TS, Hamadani M, Park SI, Flowers CR, Cohen JB. Complex karyotype in patients with mantle cell lymphoma predicts inferior survival and poor response to intensive induction therapy. Cancer. 2018;124:2306–15.
169. Eskelund CW, Dahl C, Hansen JW, Westman M, Kolstad A, Pedersen LB, Montano-Almendras CP, Husby S, Freiburghaus C, Ek S, Pedersen A, Niemann C, Raty R, Brown P, Geisler CH, Andersen MK, Guldberg P, Jerkeman M, Gronbaek K. TP53 mutations identify younger mantle cell lymphoma patients who do not benefit from intensive chemoimmunotherapy. Blood. 2017;130:1903–10.
170. Aukema SM, Hoster E, Rosenwald A, Canoni D, Delfau-Larue MH, Rymkiewicz G, Thorns C, Hartmann S, Kluin-Nelemans H, Hermine O, Dreyling M, Klapper W. Expression of TP53 is associated with the outcome of MCL independent of MIPI and Ki-67 in trials of the European MCL Network. Blood. 2018;131:417–20.
171. Menke DM, Tiemann M, Camoriano JK, Chang SF, Madan A, Chow M, Habermann TM, Parwaresch R. Diagnosis of Castleman's disease by identification of an immunophenotypically aberrant population of mantle zone B lymphocytes in paraffin-embedded lymph node biopsies. Am J Clin Pathol. 1996;105:268–76.
172. Ruchlemer R, Parry-Jones N, Brito-Babapulle V, Attolico I, Wotherspoon AC, Matutes E, Catovsky D. B-prolymphocytic leukaemia with t(11;14) revisited: a splenomegalic form of mantle cell lymphoma evolving with leukaemia. Br J Haematol. 2004;125:330–6.
173. Swerdlow SH, Williams ME. Centrocytic lymphoma: a distinct clinicopathologic, immunophenotypic, and genotypic entity. Pathol Annu. 1993;28(Pt 2):171–97.
174. Engelhard M, Brittinger G, Huhn D, Gerhartz HH, Meusers P, Siegert W, Thiel E, Wilmanns W, Aydemir U, Bierwolf S, Griesser H, Tiemann M, Lennert K. Subclassification of diffuse large B-cell lymphomas according to the Kiel classification: distinction of centroblastic and immunoblastic lymphomas is a significant prognostic risk factor. Blood. 1997;89:2291–7.

Chapter 3
Primary Cutaneous B-cell Lymphomas: FL, MCL, Differential Diagnosis

Robert E. LeBlanc

Primary Cutaneous B-Cell Lymphomas

Primary cutaneous lymphomas are non-Hodgkin lymphomas that arise in the skin. At the time of presentation, they generally do not have clinical evidence of extracutaneous disease [1]. Primary cutaneous B-cell lymphomas (PCBCL) comprise roughly a quarter of the lymphomas in this broad and heterogeneous category [2]. These lymphomas pose three main diagnostic challenges for the healthcare team: PCBCL must be distinguished from cutaneous lymphoid hyperplasias and other inflammatory diseases that mimic lymphoma, PCBCL subtypes must be distinguished from one another, and PCBCL must be distinguished from extracutaneous lymphomas that secondarily involve the skin. When a patient does not have a previous diagnosis of an extracutaneous B-cell lymphoma or has a previous diagnosis of an extracutaneous B-cell lymphoma but no tissue available for a comparative clonality assessment, a pathologist may not be able to determine whether a lymphoma is of cutaneous or extracutaneous origin. Therefore, a multidisciplinary approach to the diagnosis is necessary.

In general, PCBCL have a higher prevalence among men and among patients of middle to advanced age [3]. Per the World Health Organization (WHO) updated 2016 classification, there are three main diagnostic entities comprising this group: primary cutaneous marginal zone lymphoma (PCMZL), primary cutaneous follicle center lymphoma (PCFCL), and primary cutaneous diffuse large B-cell lymphoma, leg-type (PCDLBCL-LT) [2]. Both PCMZL and PCFCL are indolent diseases with rare exceptions. In contrast, PCDLBCL-LT is an aggressive lymphoma with a propensity to disseminate beyond the skin. Reports of skin-limited B-cell lymphomas that warrant consideration for other WHO classifications are exceptional, and less is

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known about their biologic potentials. For instance, the skin rarely hosts the initial clinical manifestations of mantle cell lymphoma. While the concept of a primary cutaneous mantle cell lymphoma has been discussed, many reported cases with clinical follow-up show that patients have occult nodal disease concomitant with their skin findings or develop extracutaneous dissemination on follow-up, which is in keeping with the multisystemic involvement attributed to this aggressive malignancy [4]. Nonetheless, it is important to consider a broad differential diagnosis for any robust cutaneous lymphoid infiltrate since a wide variety of B-cell lymphomas and other lymphocyte-rich processes can disseminate to the skin and be mistaken for primary cutaneous lymphoma. This list includes intravascular large B-cell lymphoma (IVLBCL), which is exceedingly rare but can be diagnosed on skin biopsies. The classification and our understanding of these lymphomas continue to evolve with advancements in immunobiology and molecular pathology.

Primary Cutaneous Marginal Zone B-Cell Lymphoma

Primary cutaneous marginal zone B-cell lymphoma (PCMZL) is subsumed under the WHO classification category of extranodal marginal zone lymphoma of mucosal-associated lymphoid tissue (MALT). Skin is the second most common site for MALT-type lymphoma after the gastrointestinal tract, and PCMZL is the second most common PCBCL accounting for approximately 25% of lymphomas in the category [2, 3]. An association between PCMZL and *Borrelia burgdorferi* has been established in some Western European cohorts; however, this association has not been made in North American and Asian populations [5, 6]. While the etiology is obscure for a majority of patients, an increased incidence of autoimmunity, allergies, and various gastrointestinal pathologies is diagnosed in patients with PCMZL as compared to the general population [7, 8]. Patients are twice as likely to be male than female and have a median age of 50; however, there is a wide age range from 6 to 93 years [9–12]. PCMZL can be comprised of single or multiple, clustered red to violaceous plaques or nodules. They occasionally have an annular rim of erythema [2, 9, 13]. PCMZL is more likely to arise on the trunk, upper extremities, and head and neck than elsewhere [9, 10, 14]. Patients lack constitutional symptoms, and a majority of patients have localized disease. Overall, 5-year overall survival is 97%, and it approximates 100% for patients with solitary lesions [2, 10, 15]. Dissemination beyond skin is rare with sporadic reports of nodal dissemination and exceptional reports of transformation to a large cell phenotype [9, 10, 15–17]. Approximately 93% of patients with solitary or localized lesions and 75% with broader cutaneous dissemination achieve a complete response to therapy [9]. Treatment of solitary or localized lesions can include intralesional steroids, radiotherapy, or surgical excision. Radiation has been associated with superior clearance rates [18–20]. Rate of relapse is 39% for patients with solitary or localized lesions and 77% for patients with multifocal disease [9]. Per National Comprehensive Cancer Network (NCCN) guidelines, patients receiving a putative histologic

diagnosis of PCMZL should be screened for extracutaneous disease with a workup that includes physical examination, complete blood count with differential, comprehensive metabolic panel, lactate dehydrogenase, and CT chest/abdomen/pelvis with contrast [21].

Histomorphology

There are two histologically (and likely etiologically) distinct subsets of PCMZL [8, 22]. While both comprise nodular and diffuse, bottom-heavy dermal lymphoid infiltrates that spare the epidermis and involve the subcutis (Fig. 3.1a), one subset does not undergo heavy chain class switching and resembles its extracutaneous MALT lymphoma counterparts replete with sheets of neoplastic B-cells that colonize germinal center follicles and efface adnexa. The other subset undergoes heavy chain class switching and is accompanied by a polymorphous infiltrate that is sometimes

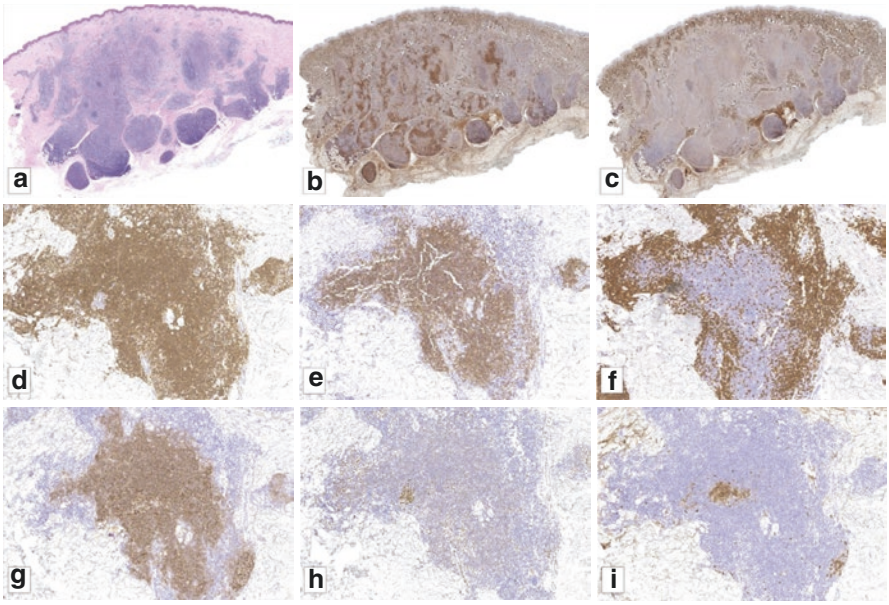

Fig. 3.1 Primary cutaneous marginal zone lymphoma. A nodular and diffuse, bottom-heavy lymphoid infiltrate spares the epidermis, occupies much of the reticular dermis, and extends into the subcutis (**a**. 10×). A kappa light chain-restricted population is evident on low power given the preponderance of kappa-positive cells (**b**. 10×) in comparison to Lambda expression (**c**. 10×). Another example illustrates colonization of a germinal center follicle by lymphoma cells that express both CD20 (**d**. 20×) and BCL2 (**e**. 20×). T-cells co-express BCL2 and CD3 (**f**. 20×) around the periphery of the infiltrate. CD21 highlights an irregular, follicular dendritic cell network with lysis at the periphery underlying the B-cell infiltrate (**g**. 20×); however, only a small, atretic residuum of the colonized follicle is revealed with BCL6 (**h**. 20×) and CD10 staining (**i**. 20×)

histologically indistinguishable from cutaneous lymphoid hyperplasia. There has been some discussion as to whether all PCMZL are bona fide lymphomas or if, in some instances, they represent an indolent clonal expansion of B-cells in the skin in the setting of T-cell-mediated B-cell hyperplasia [23]. In keeping with this hypothesis, clonal B-cells may comprise a small minority of the infiltrate in class-switched PCMZL, which is enriched for T-cells and reactive germinal center follicles. The clonal B-cells in both subtypes of PCMZL are small and can have variable morphologies including monocytoid cells, cells with cleaved nuclei, and cells with rounded nuclear contours. They may be cytomorphologically indistinguishable from the accompanying lymphocytes, particularly when they comprise a minority of the infiltrate.

Plasmacytic differentiation in PCMZL is highly variable, ranging from sparse cells scattered around the periphery of lymphoid nodules to lymphomas with exclusive plasmacytoid differentiation. The latter has previously been referred to as immunocytoma [24]. Plasma cell cytologic atypia, including binucleated cells and Dutcher bodies, can be prevalent and may serve as a clue to the diagnosis of lymphoma. Immunohistochemistry and in situ hybridization can demonstrate light chain restriction in cases with significant plasmacytic differentiation (Fig. 3.1b, c). This method offers greater sensitivity for establishing clonality in PCMZL than IgH clonality studies using PCR-capillary gel electrophoresis [11, 25]. In very rare reports, PCMZL has undergone transformation to an aggressive lymphoma characterized by blastic cytomorphology, [26] although blastoid features present in PCMZL at disease onset do not always portend aggressive behavior [27].

Ancillary Studies

As previously mentioned, there is ongoing discussion as to whether a majority of PCMZL, in light of their excellent clinical outcomes, represent a benign clonal B-cell expansion as opposed to a bona fide lymphoma [23, 28, 29]. Aiding this hypothesis is the observation that B-cell clonality has been well-documented in the setting of cutaneous lymphoid hyperplasias [30, 31]. Nonetheless, the presence of rare chromosomal translocations [32, 33] and *FAS* gene alterations [34] in a subset of PCMZL strongly supports a neoplastic origin for at least a minority of cases, as do exceedingly rare and exceptional reports of PCMZL progressing to an aggressive disease. Nonetheless, it is well-established that peripheral lymphomas can evolve in the setting of various inflammatory milieus. It is therefore not surprising that biopsies of developing lesions sometimes reveal histomorphologic findings that are diagnostically equivocal. From a strict nosological perspective, there is no single consensus on what diagnoses these borderline cases should receive or on which histologic features predict persistent growth and local recurrence. However, an unequivocal lymphoma diagnosis may provoke significant patient anxiety, even in cases where the clinical management would not differ significantly for a patient with a firm histologic diagnosis of unilesional PCMZL versus a provisional diagnosis of

cutaneous lymphoid hyperplasia with mention of features equivocal for an evolving PCMZL. Contrasting with these borderline cases, lesions of PCMZL that are diagnostically straightforward from a clinical and histopathologic perspective should be carefully distinguished from PCFCL and from cutaneous dissemination of extracutaneous lymphomas, particularly marginal zone lymphoma, chronic lymphocytic leukemia/small cell lymphoma, and mantle cell lymphoma.

The basic, nonspecific mature B-cell immunophenotype of PCMZL (BCL2+, BCL6-, CD10-) distinguishes PCMZL from PCFCL; however it cannot distinguish PCMZL from cutaneous lymphoid hyperplasia and other indolent T-cell-rich lymphoid processes. In contrast with extracutaneous marginal zone lymphomas, a number of PCMZL have been shown to be CD43 negative [35]. The marker may be especially challenging to interpret in cases of PCMZL and cutaneous lymphoid hyperplasia where CD43-positive T-cells predominate. The distinction between PCMZL and cutaneous lymphoid hyperplasia cannot always be made on histologic grounds; however, clues to a PCMZL diagnosis include dense, diffuse sheets of monotonous lymphocytes, colonization of germinal center follicles by a monomorphous B-cell infiltrate (Fig. 3.1d–i), cases demonstrating a light chain-restricted plasma cell population, the presence of cytomorphologically abnormal plasma cells, and lesions that show extensive plasmacytic differentiation. PCMZLs with extensive plasmacytic differentiation will often show decreased or absent CD20 expression but express other mature B-cell markers, including CD79a and CD19 [36]. Many of these cases express the IgG4 isotype, which is true of a majority of class-switched PCMZL; however, IgG4 expression should not be used in isolation to exclude the possibility of an extracutaneous lymphoma secondary involving the skin [37, 38]. CD5 is negative in PCMZL, except in exceedingly rare reported cases of transformed blastic PCMZL. It is therefore useful for distinguishing PCMZL from chronic lymphocytic leukemia/small cell lymphoma (CLL/SLL) and mantle cell lymphoma. It is particularly important to distinguish PCMZL from mantle cell lymphoma given the propensity of the latter to behave aggressively. A majority of mantle cell lymphomas also express Cyclin D1, which is neither expressed in PCMZL nor in CLL/SLL. Sox11, which is positive in rare mantle cell lymphomas lacking Cyclin D1 expression, may also be helpful for excluding the possibility of mantle cell lymphoma, but staining should be interpreted with caution as reactivity has been reported in a subset of marginal zone lymphomas of splenic origin [39]. In contrast with diffuse large B-cell lymphoma, PCMZL is comprised of small, mature-appearing lymphocytes and Ki-67 stains only a minority of nuclei.

Class-switched PCMZL, which can be particularly challenging if not impossible to distinguish from cutaneous lymphoid hyperplasia both clinically and histologically (Fig. 3.2), frequently expresses IgG4, does not express CXCR3, and develops in association with a T-cell helper type 2 microenvironment [8, 22, 37]. In contrast, non-class-switched PCMZL expresses IgM and is CXCR3 positive in keeping with their extracutaneous MALT lymphoma counterparts. Clinical correlations should be sought in these cases in order to evaluate for the possibility of an extracutaneous MALT lymphoma secondarily involving the skin. While routine histomorphologic features may not permit for this distinction, the translocations' characteristic of

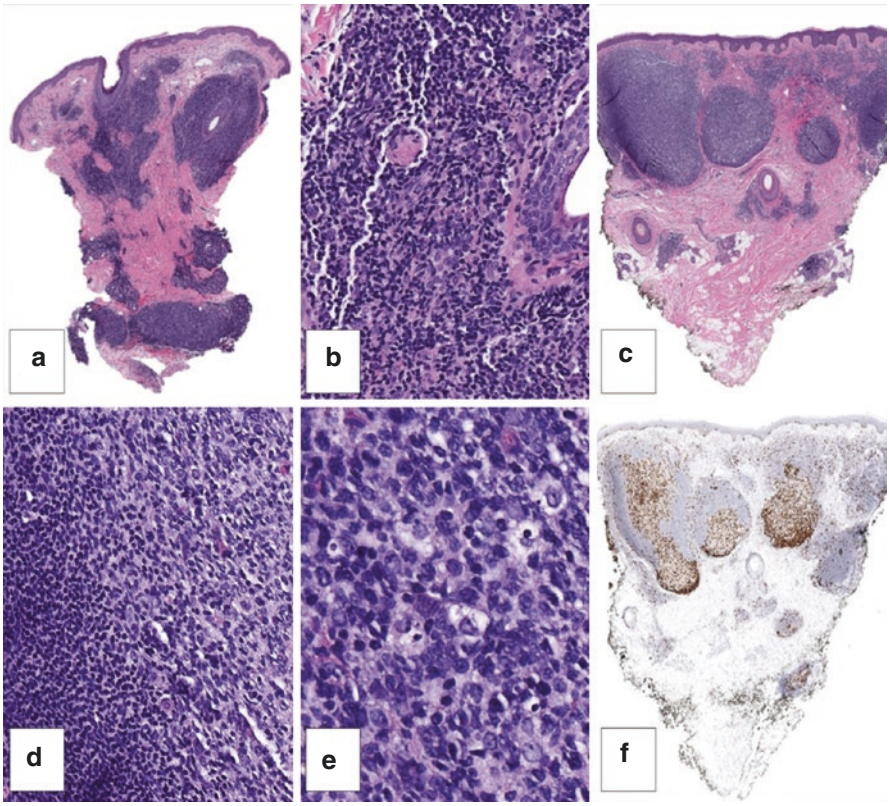

Fig. 3.2 Benign mimics of lymphoma. Pseudolymphomatous folliculitis can extend deep to the subcutis mimicking lymphoma; however, the inflammation often conforms to the shape of a hair follicle and surrounds adnexa while sparing the intervening dermal interstitium (**a**. 20×). The infiltrate is comprised of small, mature-appearing lymphocytes (**b**. 200×). Cutaneous lymphoid hyperplasia can exhibit variably sized and shaped germinal center follicles that mimic the appearance of lymphoma. Note that the follicles in this example involve only the superficial to mid-reticular dermis (**c**. 20×). Furthermore, a prominent mantle zone surrounds the follicle (**d**. 200×). Numerous tingible body macrophages are present in the germinal center in association with centrocytes and centroblasts (**e**. 400×). Ki-67 labels a majority of the germinal center B-cells with obvious polarization (**f**. 20×)

extracutaneous MALT lymphomas has a significantly lower prevalence in PCMZL. For instance, t(14;18)(q32;q31) is present in only 15% of PCMZL, [33, 40] and t(3;14)(p14.1;q32), which has a higher prevalence in thyroidal MALT lymphomas, is present in 10% of PCMZL [32].

Rare B-cell lymphomas exhibit epidermotropism with single cells. Many reported examples have an immunophenotype that is compatible with PCMZL, and interestingly, several of these rare patients share a distinct clinical presentation of recurrent crops of pink, variably pruritic papules on their torsos and extremities. Extracutaneous involvement has been documented, but these rare

cases have been associated with excellent survival approaching that of PCMZL. Some of the patients in this unusual subgroup of lymphomas have had clinical findings that could support a clinical diagnosis of splenic diffuse red pulp small B-cell lymphoma, including splenomegaly and bone marrow involvement at the time of diagnosis [41, 42]. Other patients have had no evidence of extracutaneous involvement at the time of diagnosis, supporting the idea that these cases represent a novel, de novo primary cutaneous lymphoma [43]. It has not been established whether epidermotropic B-cell lymphomas represent a heterogeneous group of lymphomas or a lymphoma with a propensity to involve the skin, spleen, and marrow in varying proportions. The nosological distinction may be an academic one since each patient with an epidermotropic B-cell infiltrate requires a comprehensive clinical evaluation for systemic disease. However, the indolent behavior of these unusual entities to date suggests that aggressive management may be unnecessary. Importantly, the clinical presentation and gross morphology of all epidermotropic B-cell lymphomas reported to date have been distinct from that of mycosis fungoides, a T-cell disease comprising the majority of cutaneous lymphomas. Although myriad clinical variants are described, mycosis fungoides generally manifests as erythematous patches with scale and arises in a photoprotected anatomic distribution. While there are rare reports of mycosis fungoides exhibiting aberrant CD20 expression, the aforementioned clinical morphology that characterizes mycosis fungoides would cast doubt on a diagnosis of B-cell lymphoma. The pattern of single cell epidermal infiltration described in epidermotropic B-cell lymphomas is also distinct from the intraepidermal lymphoid aggregates that characterize developed lesions of mycosis fungoides. Additional mature T-cell and B-cell markers should be used to confirm the lineage if there is any diagnostic uncertainty [44].

Lastly, a subset of posttransplant lymphoproliferative disease (PTLD) can have the appearance and phenotype of PCMZL. In contrast with conventional PCMZL, however, they are EBER positive, and a majority of these rare reported cases are IgA positive [45]. Additional EBV-positive PCMZL has been documented in the settings of congenital immunodeficiency, immunosuppression, and immunosenescence in patients of advanced aged [46].

Primary Cutaneous Follicle Center Lymphoma

Primary cutaneous follicle center lymphoma (PCFCL) is the most common PCBCL comprising 60% of lymphomas in the category [2]. Male patients outnumber female patients with a median age of onset of 50 years [2]. Clinically, PCFCL presents as slow-growing, smooth red-to-violaceous nodules with a tendency to arise on the head and neck, trunk, and upper extremities [2, 47, 48]. Although 80% of patients have multiple discrete lesions at the time of diagnosis, disease is generally localized [2, 47, 48]. Prognosis is excellent with posttreatment survival approaching 99% [2]. Wide cutaneous dissemination would suggest an alternative diagnosis. A minority

of patients present with large infiltrated papules and plaques on their back, and another small subset present with agminated miliary papules; however, these clinical variants do not influence prognosis [47, 49, 50]. Despite the overall indolent behavior associated with PCFCL, persistent local growth is common, and relapse occurs in a third of patients. Extracutaneous spread is rare and would raise alternative consideration for a diagnosis of follicular lymphoma secondarily involving the skin [2]. Prognosis is independent of disease multifocality with one critically important exception: PCFCL localized to the leg is associated with a marked decreased in survival approaching that of PCDLBCL-LT [47].

Since PCFCL and follicular lymphomas of nodal origin show substantial clinical, histologic, and immunophenotypic overlap in the skin, NCCN guidelines indicate that patients receiving a putative histologic diagnosis of PCMZL should be screened for extracutaneous disease with a workup that includes physical examination, complete blood count with differential, comprehensive metabolic panel, lactate dehydrogenase, and CT chest/abdomen/pelvis with contrast [21]. Treatment options for PCFCL are similar to those of PCMZL.

Histomorphology

Two predominant architectural patterns attributable to PCFCL are irregular, overlapping nodules that recapitulate germinal center follicles and diffuse sheets of cells with a germinal center phenotype. These patterns can be combined in a single lesion. PCFCL spares the epidermis, fills the dermis, and regularly extends to the subcutis. The lesional B-cells are medium-sized and resemble centrocytes and centroblasts that comprise benign germinal center follicles. In contrast with benign follicles, the follicular structures of PCFCL show a loss of polarization, an absence of tingible body macrophages, underdeveloped or absent mantle zones, and extension of neoplastic cells beyond the borders of the underpinning follicular dendritic cell networks. Neoplastic B-cells resemble cleaved centrocytes with a widely variable number of accompanying centroblasts that predominate in some cases (Fig. 3.3a–c). In contrast with nodal follicular lymphomas, however, PCFCL is not assigned a histologic grade regardless of architecture and cytomorphology [47]. Nonetheless, dense sheets of centroblasts without smaller accompanying lymphocytes, the presence of immunoblasts, significant cytologic atypia, prominent mitotic activity, and single cell necrosis are features that would challenge a diagnosis of PCFCL and raise histologic consideration for diffuse large B-cell lymphoma. PCFCL with a preponderance of centroblasts generally has small accompanying lymphocytes in contrast with the monomorphous infiltrates of PCDLBCL-LT. Dermal sclerosis is also commonly identified in PCFCL and is less often reported in diffuse large B-cell lymphomas. Rarely PCFCL demonstrates bizarre Reed-Sternberg-like cells [51, 52] or a sarcomatoid appearance with spindled B-cells dissecting through the dermal interstitium; however, these cytomorphologic variations have not been shown to effect prognosis [53–56].

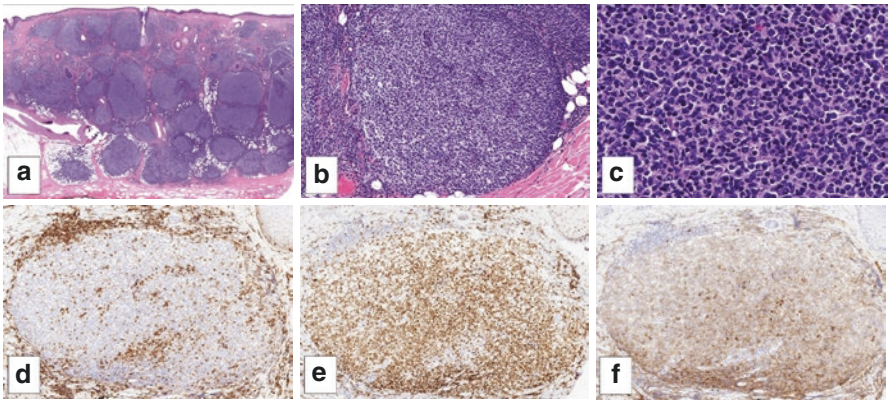

Fig. 3.3 Primary cutaneous follicle center lymphoma. Variably sized intradermal and subcutaneous nodules recapitulating germinal center follicles (**a**. 10×). However, these abnormal follicles lack polarity and mantle zones (**b**. 100×). Enlarged, neoplastic centrocytes with cleaved nuclei and scattered centroblasts with conspicuous nucleoli are present, and there is a relative paucity of tingible body macrophages (**c**. 400×). The lymphoma cells are BCL2-negative (**d**. 100×), BCL6-positive (**e**. 100×), and weakly CD10-positive (**f**. 100×)

Ancillary Studies

The three most common diagnostic conundrums entail distinguishing PCFCL from cutaneous lymphoid hyperplasias, distinguishing PCFCL from follicular lymphomas of nodal origin that secondarily involve the skin, and distinguishing PCFCL from diffuse large B-cell lymphomas. All cases of PCFCL express germinal center follicle markers, definitionally BCL6 with varied and heterogeneous coexpression of CD10 (Fig. 3.3d–f). In contrast with follicular lymphoma, however, PCFCL is more likely to be CD10 negative, particularly in cases with diffuse architecture [9, 47]. When the diagnosis is in question, it may be helpful to incorporate additional germinal center follicle markers to aid in diagnosis. In order of decreasing sensitivity, these markers include STMN1, LMO2, HGAL, and AID [57]. BCL2 can be positive or negative in PCFCL and therefore cannot reliably distinguish PCFCL from nodal follicular lymphoma. Mutually exclusive rearrangements involving *BCL2* and loss of 1p36 can be identified in both of these lymphomas, albeit the prevalence is reportedly lower in PCFCL [58, 59]. While the majority of follicular lymphomas have an associated t(14;18)(IGH;BCL2), data are conflicting on its prevalence in PCFCL, reportedly ranging from 0% to 51% [60–63]. Variation may reflect different methods used for detection. The presence of this translocation in PCFCL has been shown to correlate with BCL2 expression by immunohistochemistry, which is often faint in contrast with stronger expression evident in follicular lymphomas that secondarily involve the skin [61–63]. Limited data suggest that the detection of t(14;18)(IGH;BCL2) in a case of suspected PCFCL does not indicate a worse prognosis; however, its presence in a cutaneous lymphoid infiltrate showing strong CD10 and BCL2 coexpression should prompt consideration for clinically

occult nodal disease [63]. Deletions of 1p36 involving the CD10 locus are common in both PCFCL and follicular lymphoma and also should not be used to distinguish them [58, 64, 65].

Cases of PCFCL with a predominance of large centrocytes generally have a similar excellent response to therapy and survival [66, 67]. However rare cases of PCFCL can undergo transformation to an aggressive large cell phenotype [68]. Similarly, there are de novo primary cutaneous lymphomas that have features ambiguous for PCFCL and PCDLBCL. Ki-67 expression is low in PCFCL, and staining will reveal a loss of the germinal center polarization that is characteristic of cutaneous lymphoid hyperplasias. By comparison, Ki-67 is markedly elevated in diffuse large B-cell lymphomas, staining a majority of the lymphoma nuclei. PCDLBCL-LT generally demonstrates an activated B-cell (ABC) phenotype with expression of MUM1, FOX-P1, and IgM, which can be helpful for distinguishing it from PCFCL with a preponderance of large cells. In contrast, fewer than 30% of the B-cells comprising PCFCL express these markers [69, 70]. Kappa and lambda stains or in situ hybridization is generally unhelpful in establishing a diagnosis of PCFCL; however, IgH gene rearrangement studies can demonstrate clonality if necessary. While clonality cannot reliably distinguish lymphoma from lymphoid hyperplasia, it could potentially aid in the comparison of a diffuse large B-cell lymphoma to a prior or concomitant biopsy of PCFCL when transformation is suspected.

Primary Cutaneous Diffuse Large B-Cell Lymphoma, Leg-Type

Primary cutaneous diffuse large B-cell lymphoma, leg type (PCDLBCL-LT), is an aggressive malignancy that comprises slightly less than 20% of all PCBCL [2]. In contrast with PCMZL and PCFCL, PCDLBCL-LT has a median age of onset in the 70s and a predilection to involve women on their lower extremities [2]. Clinically, this lymphoma presents as large red-to-violaceous nodules. Despite its nomenclature, 15% of cases arise on non-leg locations; however, a unifying feature is that a majority of cases described manifest in the skin as a de novo lymphoma and share an ABC phenotype [2, 48, 66]. Altogether, PCDLBCL-LT has a 5-year disease-specific survival of 50%–70% [2]. Negative prognostic indicators include the presence of multiple lesions, age greater than 75 years, and leg involvement, which alone is associated with a 5-year disease-specific survival of 43% owing to a higher risk of extracutaneous dissemination [67]. Per NCCN guidelines, patients with a new diagnosis of DLBCL involving the skin should be staged with PET-CT, bone marrow biopsy, and peripheral blood flow cytometry in order to exclude the possibility of secondary skin involvement by an extracutaneous lymphoma, which has a higher incidence than PCDLBCL-LT, occurring in approximately 10% of patients with extracutaneous DLBCL [68, 71]. Additionally, male patients should undergo testicular ultrasonography since testicular DLBCL and PCDLBCL-LT are histomorphologically indistinguishable [72]. The mutational profile of PCDLBCL-LT

overlaps with both testicular lymphoma and primary central nervous system lymphoma (PCNSL) [73]. The standard treatment includes rituximab, combination chemotherapy, and radiotherapy [74–76]. Relapses and extracutaneous dissemination of PCDLBCL-LT are common [2, 67].

Histomorphology

Sheets of large, often monomorphous lymphoid cells fill the dermis and can involve the subcutis. The overlying epidermis is sometimes ulcerated. There are few to no accompanying small lymphocytes and granulocytes, in contrast with cases of PCFCL with a predominance of centroblasts and with other diffuse large B-cell lymphoma subtypes. The cells comprising PCDLBCL-LT show an immunoblastic, centroblastic, or mixed appearance with frequent mitoses.

Ancillary Studies

PCDLBCL-LT should be distinguished from PCFCL and extracutaneous large B-cell lymphomas whenever possible. Some cutaneous large cell lymphomas lack an ABC phenotype and present as sheets of monomorphous blasts including immunoblasts [77].

The majority of PCDLBCL-LT has an ABC phenotype demonstrable by gene expression profiling [9, 78] and exhibits a corresponding immunophenotype (MUM1+/BCL2+/CD10-). PCDLBCL-LT is further characterized by *MYD88* L265P mutations in 70% of cases [79–82]. Ki-67 highlights the vast preponderance of lesional blasts (Fig. 3.4) in contrast with the relatively low percentage of Ki-67-positive nuclei encountered in indolent PCBCL. PCDLBCL-LT commonly co-expresses BCL6. This marker is therefore of little diagnostic aid in distinguishing PCDLBCL-LT from PCFCL and from extracutaneous diffuse large B-cell lymphomas. FOX-P1, IgM, and p63-positivity in >30% of the lymphoid infiltrate can help distinguish PCDLBCL-LT from PCFCL with a preponderance of large cells [69, 70]. CD21-positive follicular dendritic cell networks are not present in PCDLBCL-LT. EBER in situ hybridization is negative in PCDLBCL-LT and should be performed to exclude an Epstein-Barr virus-associated DLBCL.

PCDLBCL-LT controlled for cases with an ABC phenotype has an exceedingly low prevalence of *MYC* rearrangements. The rearrangement has been identified in only 4.5% of such cases [78, 83–86]. Other studies permitting greater immunophenotypic variety for a diagnosis of PCDLBCL-LT have demonstrated *MYC* rearrangements in 32% of cases [87]. In general, *MYC* rearrangements are more prevalent in diffuse large B-cell lymphomas exhibiting a GCB phenotype [88]. In some case series, up to 10% of PCDLBCL-LT were described as lacking the

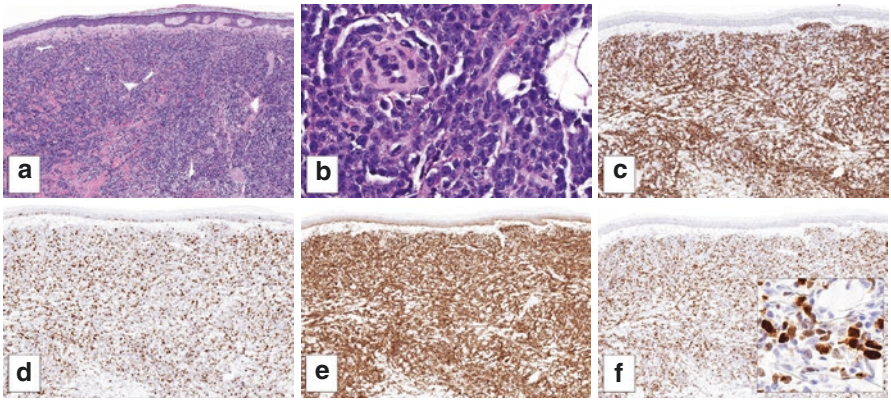

Fig. 3.4 Diffuse large B-cell lymphoma, leg type. Sheets of lymphoid cells fill the reticular dermis and spare the epidermis (**a**. 40×). A preponderance of enlarged lymphoid cells with prominent central nucleoli crowds the dermis (**b**. 400×). The lymphoma cells express CD20 (**c**. 40×), Ki-67 (**d**. 40×), BCL2 (**e**. 40×), and MUM1 (**f**. 40×, inset 400×)

requisite BCL2 and MUM1 expression to classify the lymphoma as ABC [2, 89, 90], and some cases of PCDLBCL with a BCL2-/BCL6+ immunophenotype behave aggressively in keeping with their ABC counterparts. Thus, there is ongoing discussion as to how these lymphomas are best classified [79]. In general, a diagnosis of PCDLBCL-LT should be reconsidered when the infiltrate is polymorphous with numerous accompanying small lymphocytes, an ABC immunophenotype cannot be established, and no *MYD88* L265P mutation is identified [84, 91]. Reports of double-hit translocations in PCDLBCL-LT are exceedingly rare in all studies regardless of diagnostic criteria [87]. Loss of 9p21.3 including the *CDKN2A* locus is present in 75% of cases [92–94]; however, this finding is identified in other diffuse large B-cell lymphoma subtypes [95].

Other B-Cell Lymphoma that Involve Skin

Mantle cell lymphoma is an aggressive mature B-cell malignancy that occasionally involves the skin [4, 96–100]. As noted previously, there are exceptional reports of cutaneous mantle cell lymphomas that initially manifest in the skin; however, these lymphomas have a propensity for wide dissemination and poor outcomes [4, 101]. A majority of mantle cell lymphomas are characterized by the t(11;14)(q13;q32) rearrangement involving *CCND1* and *IGH*. These lymphomas generally express Cyclin D1 by immunohistochemistry. The lymphoma comprises a monotonous infiltrate of small to slightly enlarged lymphoid cells with mildly irregular nuclear contours, coarse nuclear chromatin, small inconspicuous nucleoli, and little cytoplasm; however, both the cytomorphology and architecture can vary in the skin. Centrocyte-like, blastoid and pleomorphic cytomorphologic variants are known, and cases with

diffuse, nodular and diffuse, and perivascular architectural patterns have been described. A majority of mantle cell lymphomas express Cyclin D1, SOX11, FMC7, and CD5 by immunohistochemistry, which can help distinguish them from PCMZL and PCFCL. CD23 is negative, in contrast with CLL/SLL. CLL/SLL in the skin often manifests as exaggerated host reactions to neoplasms, arthropod exposures, and other inflammatory phenomena and infrequently as cutaneous plaques in asymptomatic patients who lack a preceding diagnosis. There are exceptionally rare cases of cutaneous CLL/SLL in patients with a normal peripheral blood count [102, 103]. Importantly, CLL/SLL can lack CD20 expression if the patient has been treated with rituximab. Other mature B-cell markers, such as CD79a and CD19, should be considered to confirm B-cell origin. Otherwise, expression of CD5, CD23, and LEF1 and the absence of staining with Cyclin D1, SOX11, and germinal center follicle markers help distinguish CLL/SLL from other mature B-cell lymphomas.

Precursor B-cell acute lymphoblastic leukemia/lymphoma (B-ALL/LBL) expresses CD10, which is a potential diagnostic pitfall; however, it generally shows a loss of CD20 in keeping with an immature B-cell phenotype. B-cell lineage can be confirmed with CD79a, which is usually retrained. B-ALL/LBL also expresses TDT. These markers should be considered to evaluate for any B-cell lymphoma exhibiting a lymphoblastic appearance, particularly in pediatric and young adult patients [104].

References

1. Willemze R, Kerl H, Sterry W, Berti E, Cerroni L, Chimenti S, et al. EORTC classification for primary cutaneous lymphomas: a proposal from the cutaneous lymphoma study Group of the European Organization for research and treatment of cancer. Blood. 1997;90(1):354–71.
2. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375–90.
3. Bradford PT, Devesa SS, Anderson WF, Toro JR. Cutaneous lymphoma incidence patterns in the United States: a population-based study of 3884 cases. Blood. 2009;113(21):5064–73.
4. Gru AA, Hurley MY, Salavaggione AL, Brodell L, Sheinbein D, Anadkat M, et al. Cutaneous mantle cell lymphoma: a clinicopathologic review of 10 cases. J Cutan Pathol. 2016;43(12):1112–20.
5. Takino H, Li C, Hu S, Kuo TT, Geissinger E, Muller-Hermelink HK, et al. Primary cutaneous marginal zone B-cell lymphoma: a molecular and clinicopathological study of cases from Asia, Germany, and the United States. Mod Pathol. 2008;21(12):1517–26.
6. Cerroni L, Zochling N, Putz B, Kerl H. Infection by Borrelia burgdorferi and cutaneous B-cell lymphoma. J Cutan Pathol. 1997;24(8):457–61.
7. Guitart J, Deonizio J, Bloom T, Martinez-Escala ME, Kuzel TM, Gerami P, et al. High incidence of gastrointestinal tract disorders and autoimmunity in primary cutaneous marginal zone B-cell lymphomas. JAMA Dermatol. 2014;150(4):412–8.
8. Edinger JT, Kant JA, Swerdlow SH. Cutaneous marginal zone lymphomas have distinctive features and include 2 subsets. Am J Surg Pathol. 2010;34(12):1830–41.
9. Hoefnagel JJ, Vermeer MH, Jansen PM, Heule F, van Voorst Vader PC, Sanders CJ, et al. Primary cutaneous marginal zone B-cell lymphoma: clinical and therapeutic features in 50 cases. Arch Dermatol. 2005;141(9):1139–45.

10. Dalle S, Thomas L, Balme B, Dumontet C, Thieblemont C. Primary cutaneous marginal zone lymphoma. Crit Rev Oncol Hematol. 2010;74(3):156–62.
11. Cerroni L, Signoretti S, Hofler G, Annessi G, Putz B, Lackinger E, et al. Primary cutaneous marginal zone B-cell lymphoma: a recently described entity of low-grade malignant cutaneous B-cell lymphoma. Am J Surg Pathol. 1997;21(11):1307–15.
12. Amitay-Laish I, Tavallaee M, Kim J, Hoppe RT, Million L, Feinmesser M, et al. Paediatric primary cutaneous marginal zone B-cell lymphoma: does it differ from its adult counterpart? Br J Dermatol. 2017;176(4):1010–20.
13. Servitje O, Gallardo F, Estrach T, Pujol RM, Blanco A, Fernandez-Sevilla A, et al. Primary cutaneous marginal zone B-cell lymphoma: a clinical, histopathological, immunophenotypic and molecular genetic study of 22 cases. Br J Dermatol. 2002;147(6):1147–58.
14. Baldassano MF, Bailey EM, Ferry JA, Harris NL, Duncan LM. Cutaneous lymphoid hyperplasia and cutaneous marginal zone lymphoma: comparison of morphologic and immunophenotypic features. Am J Surg Pathol. 1999;23(1):88–96.
15. Zinzani PL, Quaglino P, Pimpinelli N, Berti E, Baliva G, Rupoli S, et al. Prognostic factors in primary cutaneous B-cell lymphoma: the Italian study Group for Cutaneous Lymphomas. J Clin Oncol. 2006;24(9):1376–82.
16. Gerami P, Wickless SC, Rosen S, Kuzel TM, Ciurea A, Havey J, et al. Applying the new TNM classification system for primary cutaneous lymphomas other than mycosis fungoides and Sezary syndrome in primary cutaneous marginal zone lymphoma. J Am Acad Dermatol. 2008;59(2):245–54.
17. Hatem J, Schrank-Hacker AM, Watt CD, Morrissette JJ, Rubin AI, Kim EJ, et al. Marginal zone lymphoma-derived interfollicular diffuse large B-cell lymphoma harboring 20q12 chromosomal deletion and missense mutation of BIRC3 gene: a case report. Diagn Pathol. 2016;11(1):137.
18. Goyal A, Carter JB, Pashtan I, Gallotto S, Wang I, Isom S, et al. Very low-dose versus standard dose radiation therapy for indolent primary cutaneous B-cell lymphomas: a retrospective study. J Am Acad Dermatol. 2018;78(2):408–10.
19. Oertel M, Elsayad K, Weishaupt C, Steinbrink K, Eich HT. De-escalated radiotherapy for indolent primary cutaneous B-cell lymphoma. Strahlenther Onkol. 2020;196:126.
20. Christensen L, Cooper K, Honda K, Mansur D. Relapse rates in patients with unilesional primary cutaneous B-cell lymphoma treated with radiation therapy: a single-institution experience. Br J Dermatol. 2018;179(5):1172–3.
21. NCCN. NCCN Guidelines for Primary Cutaneous B-cell Lymphomas. Available from: https://www.nccn.org/professionals/physician_gls/pdf/pcbcl.pdf.
22. van Maldegem F, van Dijk R, Wormhoudt TA, Kluin PM, Willemze R, Cerroni L, et al. The majority of cutaneous marginal zone B-cell lymphomas expresses class-switched immunoglobulins and develops in a T-helper type 2 inflammatory environment. Blood. 2008;112(8):3355–61.
23. Guitart J. Rethinking primary cutaneous marginal zone lymphoma: shifting the focus to the cause of the infiltrate. J Cutan Pathol. 2015;42(9):600–3.
24. Duncan LM, LeBoit PE. Are primary cutaneous immunocytoma and marginal zone lymphoma the same disease? Am J Surg Pathol. 1997;21(11):1368–72.
25. Schafernak KT, Variakojis D, Goolsby CL, Tucker RM, Martinez-Escala ME, Smith FA, et al. Clonality assessment of cutaneous B-cell lymphoid proliferations: a comparison of flow cytometry immunophenotyping, molecular studies, and immunohistochemistry/in situ hybridization and review of the literature. Am J Dermatopathol. 2014;36(10):781–95.
26. Tsukamoto N, Kojima M, Uchiyama T, Takeuchi T, Karasawa M, Murakami H, et al. Primary cutaneous CD5+ marginal zone B-cell lymphoma resembling the plasma cell variant of Castleman's disease. Case Rep APMIS. 2007;115(12):1426–31.
27. Magro CM, Yang A, Fraga G. Blastic marginal zone lymphoma: a clinical and pathological study of 8 cases and review of the literature. Am J Dermatopathol. 2013;35(3):319–26.
28. Jaffe ES. Navigating the cutaneous B-cell lymphomas: avoiding the rocky shoals. Mod Pathol. 2019;33:96.
29. Swerdlow SH. Cutaneous marginal zone lymphomas. Semin Diagn Pathol. 2017;34(1):76–84.

30. Schmid U, Eckert F, Griesser H, Steinke C, Cogliatti SB, Kaudewitz P, et al. Cutaneous follicular lymphoid hyperplasia with monotypic plasma cells. A clinicopathologic study of 18 patients. Am J Surg Pathol. 1995;19(1):12–20.
31. Nihal M, Mikkola D, Horvath N, Gilliam AC, Stevens SR, Spiro TP, et al. Cutaneous lymphoid hyperplasia: a lymphoproliferative continuum with lymphomatous potential. Hum Pathol. 2003;34(6):617–22.
32. Streubel B, Vinatzer U, Lamprecht A, Raderer M, Chott A. T(3;14)(p14.1;q32) involving IGH and FOXP1 is a novel recurrent chromosomal aberration in MALT lymphoma. Leukemia. 2005;19(4):652–8.
33. Streubel B, Lamprecht A, Dierlamm J, Cerroni L, Stolte M, Ott G, et al. T(14;18)(q32;q21) involving IGH and MALT1 is a frequent chromosomal aberration in MALT lymphoma. Blood. 2003;101(6):2335–9.
34. Maurus K, Appenzeller S, Roth S, Kuper J, Rost S, Meierjohann S, et al. Panel sequencing reveals recurrent genetic FAS alterations in primary cutaneous marginal zone lymphoma. J Invest Dermatol. 2018;138:1573.
35. Levin C, Mirzamani N, Zwerner J, Kim Y, Schwartz EJ, Sundram U. A comparative analysis of cutaneous marginal zone lymphoma and cutaneous chronic lymphocytic leukemia. Am J Dermatopathol. 2012;34(1):18–23.
36. Geyer JT, Ferry JA, Longtine JA, Flotte TJ, Harris NL, Zukerberg LR. Characteristics of cutaneous marginal zone lymphomas with marked plasmacytic differentiation and a T cell-rich background. Am J Clin Pathol. 2010;133(1):59–69.
37. Carlsen ED, Swerdlow SH, Cook JR, Gibson SE. Class-switched primary cutaneous marginal zone lymphomas are frequently IgG4-positive and have features distinct from IgM-positive cases. Am J Surg Pathol. 2019;43(10):1403–12.
38. Brenner I, Roth S, Puppe B, Wobser M, Rosenwald A, Geissinger E. Primary cutaneous marginal zone lymphomas with plasmacytic differentiation show frequent IgG4 expression. Mod Pathol. 2013;26(12):1568–76.
39. Nakashima MO, Durkin L, Bodo J, Lin J, Quintanilla-Martinez L, Fu K, et al. Utility and diagnostic pitfalls of SOX11 monoclonal antibodies in mantle cell lymphoma and other lymphoproliferative disorders. Appl Immunohistochem Mol Morphol. 2014;22(10):720–7.
40. Streubel B, Simonitsch-Klupp I, Mullauer L, Lamprecht A, Huber D, Siebert R, et al. Variable frequencies of MALT lymphoma-associated genetic aberrations in MALT lymphomas of different sites. Leukemia. 2004;18(10):1722–6.
41. Hedayat AA, Carter JB, Lansigan F, LeBlanc RE. Epidermotropic presentation by splenic B-cell lymphoma: the importance of clinical-pathologic correlation. J Cutan Pathol. 2018;45(4):299–304.
42. Mollejo M, Algara P, Mateo MS, Sanchez-Beato M, Lloret E, Medina MT, et al. Splenic small B-cell lymphoma with predominant red pulp involvement: a diffuse variant of splenic marginal zone lymphoma? Histopathology. 2002;40(1):22–30.
43. Magro CM, Momtahen S, Coleman M, Grossman ME. Epidermotropic CXCR3 positive marginal zone lymphoma: a distinctive clinical histopathological entity potentially originating in the skin; it does not always indicate splenic marginal zone lymphoma. Dermatol Online J. 2019;25(7):13030/qt4207n83g.
44. Jullie ML, Carlotti M, Vivot A Jr, Beylot-Barry M, Ortonne N, Frouin E, et al. CD20 antigen may be expressed by reactive or lymphomatous cells of transformed mycosis fungoides: diagnostic and prognostic impact. Am J Surg Pathol. 2013;37(12):1845–54.
45. Gibson SE, Swerdlow SH, Craig FE, Surti U, Cook JR, Nalesnik MA, et al. EBV-positive extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue in the post-transplant setting: a distinct type of posttransplant lymphoproliferative disorder? Am J Surg Pathol. 2011;35(6):807–15.
46. Gong S, Crane GM, McCall CM, Xiao W, Ganapathi KA, Cuka N, et al. Expanding the spectrum of EBV-positive marginal zone lymphomas: a lesion associated with diverse immunodeficiency settings. Am J Surg Pathol. 2018;42(10):1306–16.
47. Senff NJ, Hoefnagel JJ, Jansen PM, Vermeer MH, van Baarlen J, Blokx WA, et al. Reclassification of 300 primary cutaneous B-cell lymphomas according to the new WHO-

EORTC classification for cutaneous lymphomas: comparison with previous classifications and identification of prognostic markers. J Clin Oncol. 2007;25(12):1581–7.
48. Hamilton SN, Wai ES, Tan K, Alexander C, Gascoyne RD, Connors JM. Treatment and outcomes in patients with primary cutaneous B-cell lymphoma: the BC Cancer Agency experience. Int J Radiat Oncol Biol Phys. 2013;87(4):719–25.
49. Swerdlow SH, Quintanilla-Martinez L, Willemze R, Kinney MC. Cutaneous B-cell lymphoproliferative disorders: report of the 2011 Society for Hematopathology/European Association for Haematopathology workshop. Am J Clin Pathol. 2013;139(4):515–35.
50. Gulia A, Saggini A, Wiesner T, Fink-Puches R, Argenyi Z, Ferrara G, et al. Clinicopathologic features of early lesions of primary cutaneous follicle center lymphoma, diffuse type: implications for early diagnosis and treatment. J Am Acad Dermatol. 2011;65(5):991–1000.
51. Aldarweesh FA, Treaba DO. Primary cutaneous follicle centre lymphoma with Hodgkin and reed-Sternberg like cells: a case report and review of the literature. Case Rep Hematol. 2017;2017:9549428.
52. Dilly M, Ben-Rejeb H, Vergier B, Feldis M, Toty L, Nohra O, et al. Primary cutaneous follicle center lymphoma with Hodgkin and reed-Sternberg-like cells: a new histopathologic variant. J Cutan Pathol. 2014;41(10):797–801.
53. Li L, Majerowski J, Sokumbi O. Cutaneous spindled follicle center cell lymphoma with abundant mucin: a diagnostic pitfall. J Cutan Pathol. 2020;47:394.
54. Ries S, Barr R, LeBoit P, McCalmont T, Waldman J. Cutaneous sarcomatoid B-cell lymphoma. Am J Dermatopathol. 2007;29(1):96–8.
55. Cerroni L, El-Shabrawi-Caelen L, Fink-Puches R, LeBoit PE, Kerl H. Cutaneous spindle-cell B-cell lymphoma: a morphologic variant of cutaneous large B-cell lymphoma. Am J Dermatopathol. 2000;22(4):299–304.
56. Charli-Joseph Y, Cerroni L, LeBoit PE. Cutaneous spindle-cell B-cell lymphomas: most are neoplasms of follicular center cell origin. Am J Surg Pathol. 2015;39(6):737–43.
57. Verdanet E, Dereure O, Rene C, Tempier A, Benammar-Hafidi A, Gallo M, et al. Diagnostic value of STMN1, LMO2, HGAL, AID expression and 1p36 chromosomal abnormalities in primary cutaneous B cell lymphomas. Histopathology. 2017;71(4):648–60.
58. Szablewski V, Ingen-Housz-Oro S, Baia M, Delfau-Larue MH, Copie-Bergman C, Ortonne N. Primary cutaneous follicle center lymphomas expressing BCL2 protein frequently harbor BCL2 gene break and may present 1p36 deletion: a study of 20 cases. Am J Surg Pathol. 2016;40(1):127–36.
59. Gango A, Batai B, Varga M, Kapczar D, Papp G, Marschalko M, et al. Concomitant 1p36 deletion and TNFRSF14 mutations in primary cutaneous follicle center lymphoma frequently expressing high levels of EZH2 protein. Virchows Arch. 2018;473(4):453–62.
60. Vergier B, Belaud-Rotureau MA, Benassy MN, Beylot-Barry M, Dubus P, Delaunay M, et al. Neoplastic cells do not carry bcl2-JH rearrangements detected in a subset of primary cutaneous follicle center B-cell lymphomas. Am J Surg Pathol. 2004;28(6):748–55.
61. Kim BK, Surti U, Pandya A, Cohen J, Rabkin MS, Swerdlow SH. Clinicopathologic, immunophenotypic, and molecular cytogenetic fluorescence in situ hybridization analysis of primary and secondary cutaneous follicular lymphomas. Am J Surg Pathol. 2005;29(1):69–82.
62. Streubel B, Scheucher B, Valencak J, Huber D, Petzelbauer P, Trautinger F, et al. Molecular cytogenetic evidence of t(14;18)(IGH;BCL2) in a substantial proportion of primary cutaneous follicle center lymphomas. Am J Surg Pathol. 2006;30(4):529–36.
63. Servitje O, Climent F, Colomo L, Ruiz N, Garcia-Herrera A, Gallardo F, et al. Primary cutaneous vs secondary cutaneous follicular lymphomas: a comparative study focused on BCL2, CD10, and t(14;18) expression. J Cutan Pathol. 2019;46(3):182–9.
64. Katzenberger T, Kalla J, Leich E, Stocklein H, Hartmann E, Barnickel S, et al. A distinctive subtype of t(14;18)-negative nodal follicular non-Hodgkin lymphoma characterized by a predominantly diffuse growth pattern and deletions in the chromosomal region 1p36. Blood. 2009;113(5):1053–61.

65. Schmidt J, Gong S, Marafioti T, Mankel B, Gonzalez-Farre B, Balague O, et al. Genome-wide analysis of pediatric-type follicular lymphoma reveals low genetic complexity and recurrent alterations of TNFRSF14 gene. Blood. 2016;128(8):1101–11.
66. Vermeer MH, Geelen FA, van Haselen CW, van Voorst Vader PC, Geerts ML, van Vloten WA, et al. Primary cutaneous large B-cell lymphomas of the legs. A distinct type of cutaneous B-cell lymphoma with an intermediate prognosis. Dutch Cutaneous Lymphoma Working Group. Arch Dermatol. 1996;132(11):1304–8.
67. Grange F, Beylot-Barry M, Courville P, Maubec E, Bagot M, Vergier B, et al. Primary cutaneous diffuse large B-cell lymphoma, leg type: clinicopathologic features and prognostic analysis in 60 cases. Arch Dermatol. 2007;143(9):1144–50.
68. Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS. Lymphoma incidence patterns by WHO subtype in the United States, 1992-2001. Blood. 2006;107(1):265–76.
69. Koens L, Vermeer MH, Willemze R, Jansen PM. IgM expression on paraffin sections distinguishes primary cutaneous large B-cell lymphoma, leg type from primary cutaneous follicle center lymphoma. Am J Surg Pathol. 2010;34(7):1043–8.
70. Robson A, Shukur Z, Ally M, Kluk J, Liu K, Pincus L, et al. Immunocytochemical p63 expression discriminates between primary cutaneous follicle centre cell and diffuse large B cell lymphoma-leg type, and is of the TAp63 isoform. Histopathology. 2016;69(1):11–9.
71. Sterry W, Kruger GR, Steigleder GK. Skin involvement of malignant B-cell lymphomas. J Dermatol Surg Oncol. 1984;10(4):276–7.
72. Muniesa C, Pujol RM, Estrach MT, Gallardo F, Garcia-Muret MP, Climent J, et al. Primary cutaneous diffuse large B-cell lymphoma, leg type and secondary cutaneous involvement by testicular B-cell lymphoma share identical clinicopathological and immunophenotypical features. J Am Acad Dermatol. 2012;66(4):650–4.
73. Zhou XA, Louissaint A Jr, Wenzel A, Yang J, Martinez-Escala ME, Moy AP, et al. Genomic analyses identify recurrent alterations in immune evasion genes in diffuse large B-cell lymphoma, leg type. J Invest Dermatol. 2018;138(11):2365–76.
74. Brogan BL, Zic JA, Kinney MC, Hu JY, Hamilton KS, Greer JP. Large B-cell lymphoma of the leg: clinical and pathologic characteristics in a north American series. J Am Acad Dermatol. 2003;49(2):223–8.
75. Grange F, Maubec E, Bagot M, Beylot-Barry M, Joly P, Dalle S, et al. Treatment of cutaneous B-cell lymphoma, leg type, with age-adapted combinations of chemotherapies and rituximab. Arch Dermatol. 2009;145(3):329–30.
76. Senff NJ, Noordijk EM, Kim YH, Bagot M, Berti E, Cerroni L, et al. European Organization for Research and Treatment of Cancer and International Society for Cutaneous Lymphoma consensus recommendations for the management of cutaneous B-cell lymphomas. Blood. 2008;112(5):1600–9.
77. Senff NJ, Hoefnagel JJ, Neelis KJ, Vermeer MH, Noordijk EM, Willemze R, et al. Results of radiotherapy in 153 primary cutaneous B-cell lymphomas classified according to the WHO-EORTC classification. Arch Dermatol. 2007;143(12):1520–6.
78. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004;103(1):275–82.
79. Pham-Ledard A, Cappellen D, Martinez F, Vergier B, Beylot-Barry M, Merlio JP. MYD88 somatic mutation is a genetic feature of primary cutaneous diffuse large B-cell lymphoma, leg type. J Invest Dermatol. 2012;132(8):2118–20.
80. Pham-Ledard A, Beylot-Barry M, Barbe C, Leduc M, Petrella T, Vergier B, et al. High frequency and clinical prognostic value of MYD88 L265P mutation in primary cutaneous diffuse large B-cell lymphoma, leg-type. JAMA Dermatol. 2014;150(11):1173–9.
81. Koens L, Zoutman WH, Ngarmlertsirichai P, Przybylski GK, Grabarczyk P, Vermeer MH, et al. Nuclear factor-kappaB pathway-activating gene aberrancies in primary cutaneous large B-cell lymphoma, leg type. J Invest Dermatol. 2014;134(1):290–2.

82. Mareschal S, Pham-Ledard A, Viailly PJ, Dubois S, Bertrand P, Maingonnat C, et al. Identification of somatic mutations in primary cutaneous diffuse large B-cell lymphoma, leg type by massive parallel sequencing. J Invest Dermatol. 2017;137(9):1984–94.
83. Menguy S, Frison E, Prochazkova-Carlotti M, Dalle S, Dereure O, Boulinguez S, et al. Double-hit or dual expression of MYC and BCL2 in primary cutaneous large B-cell lymphomas. Mod Pathol. 2018;31(8):1332–42.
84. Menguy S, Laharanne E, Prochazkova-Carlotti M, Gros A, Vergier B, Parrens M, et al. Challenges in assessing MYC rearrangement in primary cutaneous diffuse large B-cell lymphoma, leg-type. Am J Surg Pathol. 2020;44:424.
85. Son SM, Ha SY, Yoo HY, Oh D, Kim SJ, Kim WS, et al. Prognostic impact of MYC protein expression in central nervous system diffuse large B-cell lymphoma: comparison with MYC rearrangement and MYC mRNA expression. Mod Pathol. 2017;30(1):4–14.
86. Nosrati A, Monabati A, Sadeghipour A, Radmanesh F, Safaei A, Movahedinia S. MYC, BCL2, and BCL6 rearrangements in primary central nervous system lymphoma of large B cell type. Ann Hematol. 2019;98(1):169–73.
87. Schrader AMR, Jansen PM, Vermeer MH, Kleiverda JK, Vermaat JSP, Willemze R. High incidence and clinical significance of MYC rearrangements in primary cutaneous diffuse large B-cell lymphoma, leg type. Am J Surg Pathol. 2018;42(11):1488–94.
88. Scott DW, King RL, Staiger AM, Ben-Neriah S, Jiang A, Horn H, et al. High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements with diffuse large B-cell lymphoma morphology. Blood. 2018;131(18):2060–4.
89. Willemze R, Cerroni L, Kempf W, Berti E, Facchetti F, Swerdlow SH, et al. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. Blood. 2019;133(16):1703–14.
90. Wilcox RA. Cutaneous B-cell lymphomas: 2019 update on diagnosis, risk stratification, and management. Am J Hematol. 2018;93(11):1427–30.
91. Menguy S, Beylot-Barry M, Parrens M, Ledard AP, Frison E, Comoz F, et al. Primary cutaneous large B-cell lymphomas: relevance of the 2017 World Health Organization classification: clinicopathological and molecular analyses of 64 cases. Histopathology. 2019;74(7):1067–80.
92. Senff NJ, Zoutman WH, Vermeer MH, Assaf C, Berti E, Cerroni L, et al. Fine-mapping chromosomal loss at 9p21: correlation with prognosis in primary cutaneous diffuse large B-cell lymphoma, leg type. J Invest Dermatol. 2009;129(5):1149–55.
93. Belaud-Rotureau MA, Marietta V, Vergier B, Mainhaguiet G, Turmo M, Idrissi Y, et al. Inactivation of p16INK4a/CDKN2A gene may be a diagnostic feature of large B cell lymphoma leg type among cutaneous B cell lymphomas. Virchows Arch. 2008;452(6):607–20.
94. Dijkman R, Tensen CP, Jordanova ES, Knijnenburg J, Hoefnagel JJ, Mulder AA, et al. Array-based comparative genomic hybridization analysis reveals recurrent chromosomal alterations and prognostic parameters in primary cutaneous large B-cell lymphoma. J Clin Oncol. 2006;24(2):296–305.
95. Tsang HC, Mathew S, Magro CM. An aggressive primary cutaneous follicle center lymphoma with c-MYC translocation and CDKN2A (9p21) deletion: a case report and review of the literature. Am J Dermatopathol. 2017;39(3):e44–e9.
96. Sen F, Medeiros LJ, Lu D, Jones D, Lai R, Katz R, et al. Mantle cell lymphoma involving skin: cutaneous lesions may be the first manifestation of disease and tumors often have blastoid cytologic features. Am J Surg Pathol. 2002;26(10):1312–8.
97. Marti RM, Campo E, Bosch F, Palou J, Estrach T. Cutaneous lymphocyte-associated antigen (CLA) expression in a lymphoblastoid mantle cell lymphoma presenting with skin lesions. Comparison with other clinicopathologic presentations of mantle cell lymphoma. J Cutan Pathol. 2001;28(5):256–64.
98. Lynch DW, Verma R, Larson E, Geis MC, Jassim AD. Primary cutaneous mantle cell lymphoma with blastic features: report of a rare case with special reference to staging and effectiveness of chemotherapy. J Cutan Pathol. 2012;39(4):449–53.

99. Canpolat F, Tas E, Albayrak Sonmez A, Oktay M, Eskioglu F, Alper M. Cutaneous presentation of mantle cell lymphoma. Acta Derm Venereol. 2010;90(5):548–50.
100. Bertero M, Novelli M, Fierro MT, Bernengo MG. Mantle zone lymphoma: an immunohistologic study of skin lesions. J Am Acad Dermatol. 1994;30(1):23–30.
101. Hrgovic I, Hartmann S, Steffen B, Vogl T, Kaufmann R, Meissner M. Cutaneous involvement as a rare first sign of systemic mantle cell lymphoma: a case report and review of the literature. Mol Clin Oncol. 2016;4(5):728–32.
102. Fulton E, Alapat D, Mabry A, Gao L, Shalin SC. CLL/SLL with initial presentation as a longstanding cutaneous plaque: a rare case. J Cutan Pathol. 2016;43(8):717–21.
103. Tapia G, Mate JL, Fuente MJ, Navarro JT, Fernandez-Figueras MT, Junca J, et al. Cutaneous presentation of chronic lymphocytic leukemia as unique extramedullar involvement in a patient with normal peripheral blood lymphocyte count (monoclonal B-cell lymphocytosis). J Cutan Pathol. 2013;40(8):740–4.
104. Lin P, Jones D, Dorfman DM, Medeiros LJ. Precursor B-cell lymphoblastic lymphoma: a predominantly extranodal tumor with low propensity for leukemic involvement. Am J Surg Pathol. 2000;24(11):1480–90.

Chapter 4
Follicular and Mantle Cell Lymphomas: Technical and Interpretive Considerations; Karyotyping, FISH, Chromosomal Microarray, Sequencing, B Cell Clonality, Minimal Residual Disease

Eric Y. Loo

Introduction: Genetic Testing and Lymphomas

The abnormal genetic mechanisms underlying cancer biology provide fundamentally important insights into disease development, behavior prognostication, and means for targeted intervention. Follicular and mantle cell lymphomas each have their own major oncogenic drivers, but they also have a fairly substantial array of additional somatic mutations that contribute to disease pathobiology. If the various site- and age-specific subtypes are included, the spectrum of genetic abnormalities becomes quite considerable. Genetics as related to disease biology has been covered in other chapters and will not be the primary focus here. Instead, this portion of the text will focus on technical and interpretive considerations as related to the clinical genetic testing of follicular and mantle cell lymphomas.

A tremendous amount of work has gone into clarifying the genetic determinants of disease, and a large body of knowledge for lymphoma genetics has been accumulated. However, genetic studies have become of secondary importance in the present clinical workup of lymphomas. A definitive diagnosis can usually be rendered with just the evaluation of the tumor's microscopic appearance and confirmation of an abnormal phenotype by flow cytometry and/or immunohistochemistry. If genetic testing is ordered for a lymphoma, it is often after a diagnosis has been rendered and only for a few select subtypes or for cases that have failed to respond well to conventional therapies. In contrast, upfront genetic evaluation is standard for many myeloid malignancies, often directly influencing the diagnosis, prognosis, and therapy.

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That being said, the relatively recent proliferation in new testing modalities has revitalized general interest in the genetic interrogation of lymphomas. Conventional chromosome analysis and fluorescence in situ hybridization (FISH) still have a firm role in the clinical laboratory. However, the additional genetic abnormalities that we can now interrogate for necessitate a relational interpretation of many data points. A "clear-cut" binary conclusion may be more difficult to attain, but determining the genetic patterns driving an individual's disease is the key to optimizing personalized therapy.

Cytogenetic Methodologies

Technical Considerations

Conventional Chromosome Analysis

Conventional cytogenetic analysis, also generically referred to as "karyotyping" (KT), is complex and technically demanding. A number of preanalytic variables should be considered when negative karyotype results appear inconsistent with the morphologic and clinical findings. A karyotype presents an overview of the whole genome, typically by visual inspection of Giemsa-stained (so-called G-banded) metaphase chromosomes. This testing methodology is useful for detecting structural and numerical chromosome aberrancies. However, the evaluation is "low-resolution," and an abnormality affecting at least five million base pairs is needed to create a microscopically visible chromosome alteration. Smaller abnormalities, such as gene sequence mutations or small insertions/deletions, would go undetected.

Conventional cytogenetic analysis requires acquisition of mitotic cells from live tissue culture; thus, adequate sampling and eliciting sufficient tumoral cell growth are crucial for trustworthy results. Cultures should be processed as soon as possible, ideally within 24 hours of collection. For blood and bone marrow, specimens are collected into a "green-top" sodium heparin tube. Although "lavender-top" tubes are typically used for hematological testing, the EDTA anticoagulant used in these containers inhibits growth in culture. Solid tissue biopsies should be collected into a sterile container with enough cytogenetic culture media for complete sample submersion (multiple types are commercially available). Sterile saline is a suboptimal choice, but is preferable to the tissue desiccation that would result from transport in an otherwise empty sterile container. Wrapping a biopsy specimen in sterile gauze is not helpful and will accelerate tissue desiccation and death. Exposure to any fixative solution, such as formalin, would preclude karyotyping studies. The serum in blood and marrow is buffered, and these samples can be transported at room temperature or above 4 °C. On the other hand, soft-tissue specimens should be transported on ice until culture set-up; this slows tissue-degrading enzyme activity and microbial growth.

It is critically important to inform the laboratory if there is clinical concern for lymphoma. Tissues require different environmental conditions for optimal growth in culture. Many lymphomas have a low mitotic index and tend to produce poor-quality metaphases; thus, special processing is needed to optimize chromosome preparation. Multiple cultures with different harvest dates are typically set up for suspected lymphomas, including unstimulated and B-cell mitogen-stimulated arrangements. A suboptimal culture set-up can result in false negative results due to lack of neoplastic cell growth or overgrowth of background normal cells.

Fluorescence In Situ Hybridization (FISH)

FISH analysis is carried out by hybridizing fluorescently labeled DNA probes to interphase or metaphase cells and subsequently evaluating the signal pattern by fluorescence microscopy. This methodology is ideally used in conjunction with chromosome analysis when genetic studies are indicated. The main benefit of FISH is that it can be performed on interphase cells in both live and preserved tissue; live mitotic cells are not required for analysis. In addition, FISH studies are semi-quantitative in that a percentage of abnormal cells is reported from 200 or more evaluated nuclei and often have better turnaround times when compared to conventional chromosome analysis. The key limitation is that this modality is targeted, utilizing specific pre-selected probes against rearrangements or aneuploidy that are thought to have relevance for the disease entity.

FISH can be applied to a wide range of cellular preparations such as fresh or cultured cells, banded slides, air-dried blood or marrow smears, and frozen or paraffin-embedded tissues sections. Although formalin fixation does not preclude FISH analysis, exposure to other chemicals during tissue processing may damage nucleic acids and be prohibitive for FISH. Common examples include alternative zinc- or mercury-based tissue fixatives and many decalcification solutions. EDTA is the only acceptable agent that can be used when both decalcification and genetic studies are necessary for a biopsy sample. However, the rate of EDTA decalcification is slow and will usually delay specimen processing.

Although FISH is typically not ordered in the workup of low-grade follicular lymphoma, probes to confirm *IGH/BCL2* fusion and other *BCL2* gene rearrangements are commonly available. In cases of de novo high-grade B-cell lymphoma or concern for high-grade transformation of follicular lymphoma, FISH panels to evaluate for rearrangements involving the *MYC*, *BCL2*, and *BCL6* are commonly used. Pathologic breakpoints affecting the *MYC* gene are widely distributed and may potentially be missed depending on the sites of probe coverage. FISH studies may also be used to evaluate for *IRF4/DUPS22* rearrangement, an abnormality seen in the uncommon "large B-cell lymphoma with *IRF4* rearrangement" that is more common in younger patients and has morphologic similarities to high-grade follicular lymphoma and pediatric-type follicular lymphoma [1].

FISH probes for t(11;14)(q13;q32) are available, but testing is typically not required in the histologic workup of mantle cell lymphoma. However, probes to

evaluate for the *CCND1/IGH* rearrangement are commonly included in FISH panels for chronic lymphocytic leukemia to exclude the rare cases of mantle cell lymphoma with a leukemic presentation. A subset of the rare t(11;14)-negative mantle cell lymphomas have reportedly been found to carry *CCND2* translocations, and commercial FISH probes are available for clinical application.

Chromosomal Microarray

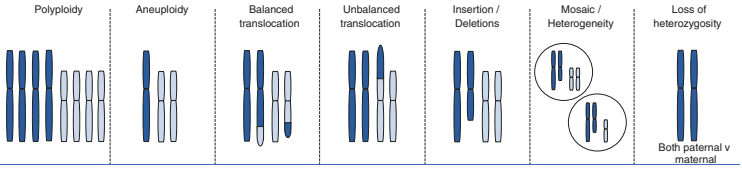
Chromosomal microarray analysis is a molecular cytogenetic method used to scan whole genomes for copy number variations, and the relative ploidy level of the test sample DNA is compared to a reference sample or reference DNA database. Compared to G-banded chromosome analysis, no cell cultures are needed, and the resolution for detection of copy number abnormalities is improved. Differing from FISH analysis, probe selection is not an issue, and no prior knowledge of a chromosomal aberration is required. The limitations of microarray testing will vary with the methodology used. The average resolution of the technology will range from tens to hundreds of kilobases and thus will not detect small sequence variants or indels within a single gene, and arrays are not well suited for the detection of balanced chromosomal rearrangements. Low-level mosaicism below 20–25% may not be detected. Tetraploidy and copy number aberrancies in regions that are not represented on the platform cannot be detected.

The main types of analyses in clinical use are array-based comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) arrays. While both will detect copy number changes, SNP arrays will also detect copy-neutral changes including loss of heterozygosity (LOH), uniparental disomy, and identity by descent. In aCGH, patient/tumor and reference DNA are labeled with green and red fluorochromes, reference DNA is fixed to a microarray grid to which patient DNA is hybridized, and the array is analyzed with software to determine differences in copy number as reflected by differences in green and red fluorescence intensity. Reference/control DNA is not used in SNP arrays; rather, hundreds of thousands of allele-specific oligonucleotide SNP probes are fixed within a microarray grid, patient DNA is hybridized to the probes, various methods employing fluorescence are used to identify binding, and fluorescent intensity is evaluated. There are two types of graphical data generated by the SNP array software. First is the log R ratio, which normalizes the total signal intensities to identify copy number changes. Each SNP locus usually will have two alleles, and the second graph plots the allelic intensity ratios for all individual loci.

The care provider ordering microarrays for oncology must be very mindful of preanalytic specimen limitations. Typically these assays will be performed on tumor DNA isolated from formalin-fixed paraffin-embedded (FFPE) tissues. The FFPE tissue blocks used for study must have a minimum of 25% tumor (by number of

nuclei), but should ideally contain >50% tumor content given the poor reproducibility of tumor estimates in clinical practice [2, 3]. If the corresponding H&E tissue section indicates ≤50% tumor content, microdissection may be attempted to bolster the proportion of neoplastic cells. The risk of false negative results is significant if estimated tumor proportions drop below 25%, and mosaic populations or subclonal heterogeneity may not be detected. As with other genetic studies, tissues fixed in heavy-metal fixatives or status-post decalcification are not acceptable. Highly proliferative tumors may also suffer from coagulative necrosis with degradation of nucleic acids. Correct specimen selection has critical implications for downstream testing; thus, this aspect of testing should be performed by a pathologist that is familiar with cytogenomic testing techniques.

As long as the proportion of tumor cells in the analyzed sample is sufficiently high, copy number variants and losses or gains of heterozygosity are readily detectable. Copy-neutral LOH will generally be due to monoallelic deletions, with the lost segment being replaced by the same region of its homologous chromosome [4, 5]. Consequences of losing one allele and duplication of a remaining mutated allele are functionally similar to a homozygous mutation. Copy-neutral LOH is a recurrent event in lymphomas and may affect a variety of genes, including tumor suppressors like TP53 [6]. However, determination of pathologic significance may be difficult as multiple gene may localize to an aneuploid gene region. Interpretation of complex chromosomal changes is a recurring dilemma in both traditional karyotyping studies and microarray analysis. Many aberrations found by microarray may be unique to a particular case and not a recurring disease-related change. These one-off copy number variants of uncertain clinical significance are found with higher frequency using microarray when compared to conventional cytogenetics and pose a significant interpretive challenge (Fig. 4.1).



Technique	Resolution	Appropriate for Detection						
Karyotype	>5 Mb	+	+	+/ –	+/ –	+/ –	+	–
FISH†	50 kb	+	+	+	+	+	+	–
CGH array	100 kb	–	+	–	+	+	+/ –	–
SNP array	10-20 kb	–	+	–	+	+	+	+

Fig. 4.1 Suitability of different cytogenetic techniques in relation of abnormality type: (+) indicates that the abnormality should be detected, (−) indicates that the abnormality would likely be missed, and (+/−) indicates that the abnormality may be detected (karyotype, if of sufficient size; CGH, additional studies may be needed). †Detection of abnormalities by interphase FISH is contingent on probe selection

Interpretive Considerations

Follicular Lymphoma

The cytogenetic characterization of lymphomas quickly proliferated after chromosomal banding techniques were introduced by Caspersson et al. in 1968 [7]. About 40 years ago in 1979, the t(14;18)(q32;q21) *IGH/BCL2* translocation was discovered [8, 9]. Although this genetic abnormality is classically taught and ascribed to be associated with follicular lymphoma, in most cases it is not critical to make a disease diagnosis. This is evidenced experientially by current medical practice; karyotyping studies are uncommon in present-day lymphoma workups, and immunophenotypic confirmation of a morphologic diagnosis usually renders genetic evaluation for these rearrangements superfluous.

Although t(14;18)(q32;q21) is generally considered the genetic hallmark of follicular lymphoma, it cannot be used to diagnose follicular lymphoma alone, it has no correlation with clinical outcomes [10], and it must be interpreted in the context of tumor histology. Somatically acquired *IGH/BCL2* gene rearrangement is not diagnostically specific and can also be found in 30% of diffuse large B-cell lymphomas, in many otherwise healthy older adults, and rarely in chronic lymphocytic leukemia and other lymphoproliferative disorders [1, 11, 12]. The translocation is more frequent in grade 1 to 2 follicular lymphoma than in grade 3 (particularly grade 3B) and can be found in most cases of duodenal-type follicular lymphoma [13, 14]. It is extremely rare in adolescent/childhood cases younger than 14 years and pediatric-type follicular lymphoma and is uncommon in most cases of primary cutaneous and non-cutaneous extranodal disease [1, 15, 16].

Additionally, the limited resolution of conventional cytogenetic analysis is unable to distinguish molecularly distinct t(14;18) rearrangements that appear to be cytogenetically identical. This issue is exemplified by the t(14;18)(q32;q21) resulting in an *IGH/MALT1* gene rearrangement, an abnormality found in extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) [1]. Both *BCL2* and the *MALT1* genes are found within the 18q21 chromosomal band, and higher-resolution FISH or molecular methods of assessment would be required to differentiate these rearrangements. Though there are multiple molecular methods that can detect *BCL2* translocations, FISH remains a robust and the most widely used method of assessment [17].

In follicular lymphoma cases that are negative for the classic t(14;18) *IGH/BCL2* rearrangement, complex/variant IGH/*BCL2* translocations and *BCL2* rearrangements with genes other than *IGH* have been described. Some of these variants may be cryptic to conventional G-banded chromosome and PCR analysis but detectable by FISH [18]. Translocations involving the immunoglobulin light chains genes have been described as notable non-*IGH* fusion partners. These appear to primarily involve the 5′ end of *BCL2*, similar to Ig light chain/*MYC* translocations, and may also be seen in other non-Hodgkin B-cell lymphomas [18–20]. Immunoglobulin light chain rearrangements are rare, and the clinical implications are not clearly defined. *BCL6* at 3q27 is the only other gene that is recognized to have recurrent

translocations in follicular lymphoma. These are found in 5–15% of follicular lymphomas and have significantly higher prevalence in grade 3B cases [21, 22]. *BCL6* rearrangements are not diagnostically specific for follicular lymphoma and may be found in 15–30% of de novo diffuse large B-cell lymphomas [1]. *BCL2* and *BCL6* gene rearrangements are not mutually exclusive; translocation of both genes may be found in around 10% of cases, but this finding is of uncertain clinical significance [23].

Translocations involving the *MYC* gene at 8q24 may rarely be found at the time of diagnosis in follicular lymphoma, but it is not considered to be primary driver of pathogenesis. However, rearrangements of *MYC* are often found in follicular lymphoma as a secondary abnormality at disease progression [24]. *MYC* gene rearrangements may involve *IGH*, as well as Ig light chains *IGK* and *IGL*; thus, *MYC* break-apart probe FISH studies are typically performed along with *MYC/IGH* rearrangement probes. Other genetic alterations that have been associated with high-grade transformation include alterations of DNA damage response through loss of *CDKN2A/B* and *TP53* [25]. Rearrangement at chromosomal bands 1q21-23 has also been described in a subset of follicular lymphoma during disease progression, and the *FCGR2B* gene is affected in cases with t(1;22)(q22;q11) [26], *FCGR2B* encodes the IgG Fc receptor, and aberrancies likely contribute to progression through resultant deregulation of B-cell proliferation.

Overt follicular lymphoma appears to arise from a clonally related follicular lymphoma in situ, and the in situ lesion generally has no or few secondary copy number alterations when evaluated by CGH and lower levels of DNA methylation in genes recurrently methylated in overt lymphoma [27]. Thus, the t(14;18) rearrangement is often viewed as a founding aberrancy, and subsequent acquisition of various potential driver abnormalities results in the progression of an in situ lesion to fully manifested lymphoma. The interpretation of karyotypic abnormalities can be daunting; about 90–95% of follicular lymphomas will carry other genetic aberrancies in addition to the t(14;18) translocation, and most will have complex karyotypes with an average of about five other genetic abnormalities [1, 28]. Cases with dysregulation of *CDKN2A/B* and/or *TP53* will typically have even greater chromosomal complexity, averaging ten chromosomal abnormalities in addition to t(14;18) on karyotype and twice as many copy number aberrancies when interrogated by chromosomal microarray [25, 28]. Additionally, an established follicular lymphoma clone often shows parallel evolution of subclonal neoplastic populations. d'Amore et al. found divergence of genetic aberrancies in 26% of the same and 63% of sequential biopsies with follicular lymphoma [29]. As the genetic profile of the dominant clone may change over time, it may be helpful to assess the disease genetics periodically, rather than only at the time of diagnosis.

Common karyotypic abnormalities include rearrangements involving chromosomes 1, 2, 4, 5, 13, and 17; gains of chromosomes X, 1, 3q27, 6p, 7, 8, 12q, and 18q; and losses of 1p, 6q 10q, and 17p [1, 10, 28, 29]. While the t(14;18) rearrangement does not correlate with clinical outcomes, some associations have been found with other karyotypic abnormalities in follicular lymphoma. Patients with deletions of chromosome regions 1p21-22, 6q23-26, or 17p and gains of 5, 12, 18q, 19, 20,

and 21 have been found to have significantly shorter survival [10, 29–32]. Gains in the X chromosome in male patients have also been correlated with worse overall survival [28]. Microarray studies have confirmed recurrent copy number aberrancies in many of the chromosomal regions with imbalances on karyotype, but generally with much higher sensitivity [33]. Microarray work has also revealed that deletions of chromosomal regions 1p36.22-p36.33 and 6q21-q24.3 are significant prognostic markers for disease transformation, independent of the international prognostic index (IPI) score [33]. No single event appears to be linked to aggressive lymphoma transformation in follicular lymphoma, and multiple routes within the diverse mutational landscape appear possible [34].

Chromosome region 1p36 is a commonly affected region in all follicular lymphomas. When analyzed by chromosomal microarray, loss of heterozygosity at 1p36.32 through deletion or acquired uniparental disomy may be found in about 25–50% of all cases, representing the most common secondary cytogenetic event in follicular lymphoma overall [35, 36]. A number of studies have associated deletion 1p36 loss with predisposition to high-grade transformation with high proliferation rates [33, 37, 38]. A number of candidate genes reside in this area including *TNFRSF9/14/18/25* genes (members of the tumor necrosis factor (TNF) receptor superfamily), *CDK11B* (aka *CDC2L1*, a cell cycle protein), and *PRDM16* (a zinc finger transcription factor) [33, 39]. Deletions of 1p36 have also been described as a recurrent finding in a subset of low-grade t(14;18)-negative nodal follicular lymphomas with a predominantly diffuse growth pattern [38]. This subset has been described as occurring more frequently in the inguinal nodes, positive co-expression of CD23, a very favorable prognosis, and frequent *TNFRSF14*, *CREBBP*, and *STAT6* gene sequence mutations [40, 41]. Some other recurrent chromosomal alterations in this t(14;18)-negative subset include gains of 1q, 2p, 3q, 6p, 9q, and 12q and losses in 4q, 11q, and 16 [40]. 1p36 deletion and co-occurring *TNFRSF14* mutations are also associated with the development of a subgroup of primary cutaneous follicle-center lymphoma [42].

Chromosome 6q abnormalities may be found in multiple B-cell neoplasms, including acute lymphoblastic leukemia, chronic lymphocytic leukemia, multiple myeloma, and diffuse large B-cell lymphoma, with involvement of 6q23-26 seen in 10–40% of B-cell lymphomas. Deletions in this region are a very common secondary abnormality in follicular lymphoma, are found by conventional chromosome analysis in 15% of cases and by microarray in about 30% of cases, and are often associated with a worse overall prognosis [10, 30, 33, 36]. Multiple regions in 6q may be deleted in follicular lymphoma, and specific loci with pathologic significance are still unclear, though 6q16, 6q21, 6q23, and 6q25-27 have been described as recurrent areas of interest [30, 33, 43]. *TNFAIP3* and *PERP* localize to 6q23 and have been implicated as genes of interest [33, 35]. *TNFAIP3* encodes a zinc finger protein and ubiquitin-editing enzyme that has been shown to inhibit NF-kappa B activation as well as TNF-mediated apoptosis. The *PERP* gene, a *TP53* apoptosis effector, induces TP53-mediated apoptosis and loss could promote tumor growth. Deletions at 6q25-27 and 6q21 were found in cases with and

without t(14;18), loss of 6q23 was typically not found in conjunction with t(14;18), and cases with loss of 6q21 have been found more often in cases with high-grade morphology [30].

Mantle Cell Lymphoma

The t(11;14)(q13;q32) *CCND1-IGH* translocation is the genetic hallmark of mantle cell lymphoma, first described by Van Den Berghe et al. about 40 years ago in 1979 [44]. The t(11;14) is much more specific, found in >95% of mantle cell lymphoma cases, and is frequently used diagnostically if arising in the right clinical context [1]. Similar to the t(14;18) translocation, t(11;14) by itself is not diagnostically specific and may be found in the context of multiple myeloma and rarely in other B-cell neoplasms [45]. Additionally, t(11;14) is like t(14;18) in that it is a weak oncogene that can be found in the blood of 1–2% of otherwise healthy persons and requires additional oncogenic events to establish a fully manifested lymphoma [46–48]. Rare cases may have variant translocations of *CCND1* with the immunoglobulin light chain genes. The small subset (<5%) of cyclin D1 and t(11,14)-negative cases overlap with conventional cases in morphology, phenotype, global gene expression profile, and secondary genetic abnormalities, and the WHO has recognized these to be the same disease entity [1, 49–51]. Many of these cases demonstrate high expression of cyclin D2 or D3, and some cases have been found to carry t(2;12)(p12;p13) which fuses cyclin D2 to the IgK light chain locus [52].

Extensive genetic workups for mantle cell lymphoma are generally uncommon. It is atypical in current clinical workflows for fresh tissue to be submitted for karyotyping studies, and in most cases, evaluation of morphology with immunohistochemical confirmation is generally sufficient to make a disease diagnosis. In cases with an unusual clinical presentation or immunophenotype, FISH studies are generally performed in an attempt to confirm presence of the t(11;14) translocation. This is generally the extent to which most cases of mantle cell lymphoma are presently genetically evaluated, but it certainly does not reflect the variety of aberrancies that underlie the process of lymphomagenesis.

Mantle cell lymphoma has a high number of non-random secondary chromosomal imbalances, and this secondary genetic event profile is essentially indistinguishable between conventional and cyclin D1-negative cases [51]. Conventional chromosome analysis will reveal secondary aberrations in about 60% of cases [53]. Common secondary abnormalities include gains of 3q26 (30–50% of cases), 7p21 (16–34%), 8q24 (16–36%), 12, and 15q and losses of 1p13-31 (29–52%), 6q23-27 (23–38%), 8p23-pter, 9p21-pter (18–31%), 11q21-q23 (21–59%), 13q11-13 (22–55%), 13q14-34 (43–51%), and 17p13 (21–45%) [1, 51, 54, 55]. High-level amplifications with overexpression are found in 36% and have been described in a variety of different regions; 11q13.3-q13.5, 13q31.3, and 18q21.33, which, respectively, contain *CCND1*, *MIR20A* (aka *C13orf25*), and *BCL2*, have been described as the most recurrently affected regions [56]. However, 8q24 (*MYC*), 10p12.2 (*BMI1*), and 12q13 (*CDK4*) have also been reported as other commonly amplified regions

[55, 57]. In addition to copy number aberrancies, copy-neutral loss of heterozygosity can be found in up to about 60% of cases analyzed by SNP array, typically involving regions where copy number losses are also found [1, 56, 58].

As secondary genetic aberrancies are not commonly evaluated, they are not generally used in prognosis calculations such as the mantle cell lymphoma international prognostic index (MIPI) score. However, a number of these abnormalities have been reported to correlate with clinical behavior, though not all appear independent of the proliferative fraction. Abnormalities that seem to be independent and consistent markers of adverse outcomes include increasing karyotypic complexity, gains of 3q, losses of 9p (including *CDKN2A* gene locus), losses of 9q21–q32, gains of 12q, and losses of 17p (*TP53*) [1, 51–55]. *BCL6* gene rearrangements resulting in BCL6 expression have been described; this may potentially cause diagnostic confusion, but does not appear to have prognostic significance [59]. *MYC* gene rearrangement is extremely rare at time of diagnosis, but is associated with disease progression/transformation and an aggressive clinical course [21, 60].

Some secondary genetic findings also appear to correlate with histologic findings. Using array CGH, Beà et al. found that blastoid mantle cell lymphoma carried a higher number of chromosomal imbalances, averaging 10.1 ± 4.89 per case, versus a mean of 3.87 ± 3.08 in classic morphologic subtypes [54]. Blastoid/pleomorphic variants also display a higher frequency of 1p and 17p deletions and 10p alterations [53]. Additionally, 80% of the pleomorphic variant, 36% of the blastoid variant, and <10% of conventional mantle cell lymphoma were found to demonstrate a tetraploid chromosome compliment [61]. Secondary abnormalities that were more often seen in leukemic presentations of mantle cell lymphoma included deletions at 8p21.3 (including loss of *TRAIL* gene locus, TF-related apoptosis-inducing ligand), gain of 8q24.1 (*MYC* gene locus), and deletions at 13q14; and gains of 3q and losses of 10p and 17p were associated with a more marked leukocytosis [55, 62, 63].

Molecular Methodologies

Technical Considerations

Biopsy Sample and Nucleic Acid Extraction

Although extremely versatile, the success of molecular diagnostic techniques depends on multiple variables including specimen quality, ratio of tumor to non-neoplastic cells, and also the quality and amount of nucleic acid required for the assay in question. A wide variety of specimens are amenable to interrogation by PCR-based molecular techniques including blood components, bone marrow, body fluids, tissue biopsies/aspirates, and paraffin-embedded tissues treated with select fixatives. Some laboratories may offer services to enrich tumor content, through either microdissection of cytologic or histologic slide preparations,

immunomagnetic cell separation, or other technologies. If molecular evaluation must be delayed due to prior authorization or other reasons, DNA or RNA can be effectively extracted, stabilized, and stored for later application.

The ordering physician should be cognizant of various issues that may limit a biopsy sample's testing utility. When liquid blood or marrow is submitted for tumor analysis, ancillary test results (e.g., CBC with differential, bone marrow report, flow cytometry results, etc.) should be cross-referenced to determine if the percent involvement by neoplastic cells in the sample is appropriate for the desired assay's sensitivity limits. Formalin is the primary fixative used for most surgical pathology applications, and formalin-fixed paraffin-embedded (FFPE) tissues are a primary specimen type submitted for genetic workup of lymphoma. FFPE offers the advantage of ready availability and straightforward assessment of tumor percentage. However, formalin-related nucleic acid fragmentation and degradation can lead to poor-quality unamplifiable DNA and/or RNA. Other factors which may affect nucleic acid quality include prefixation ischemic time, length of exposure to fixative, and age of the archived paraffin-embedded tissue block. Lymphomas of high histologic grade or increased proliferative capacity may often show regions of coagulative necrosis, and this may be a particularly limiting factor in small biopsies. While FFPE is generally acceptable for many clinical applications, it is generally not acceptable for tests requiring long fragment sizes, and small needle biopsies may suffer insufficient specimen quantity. Although RNA is less stable than DNA, FFPE is generally still suitable for RNA-centered evaluations, such as PCR-based detection of fusion genes [64].

When compared to general surgical pathology specimens, tissues for hematopathologic workup are more often exposed to alternative chemical fixatives or substrate modifying agents. Additives, such as mercury, zinc, or picric acid, have been commonly used to enhance preservation of lymph node and marrow morphology. However, these chemical additives render tissues unsuitable for molecular testing though inhibition of PCR or degradation of nucleic acids [65]. Decalcification agents used to process bone marrow core biopsies for histologic evaluation are typically strong acids, buffered acids, or calcium chelators combined with acids; and the majority of these also degrade nucleic acids and render the tissue unsuitable for molecular testing. The chelating agent ethylenediaminetetracetic acid (EDTA) is an exception and is gentle enough to allow for molecular testing [66]. The EDTA decalcification process is slow and may extend the processing time from hours to days, but sonication may help to improve workflow [67]. Bone marrow clot sections, while frequently suboptimal for morphologic evaluation, are usually more amenable than trephine sections for genetic testing.

Sequencing Technologies

The concept of DNA sequencing, identifying the nucleotide order in nucleic acids, is straightforward. However, the existing methods for achieving desired end-sequencing results are numerous, diverse in approach, and technically

complex. In 1977, Frederick Sanger's innovative dideoxynucleotide (ddNTP) "chain-termination" technique allowed for the emergence of "first-generation" sequencing, nowadays generally referred to as "Sanger sequencing" [68]. Originally dependent on manually reading band sequences in polyacrylamide gels, this method was semi-automated through replacement of radiolabeling with fluorophore-labeled ddNTPs and nucleotide fragment separation by capillary electrophoresis. This technique is still used to interrogate single genes for suspected point and insertion/deletion sequence variants, but the sensitivity of detection is relatively limited to a variant clone proportion of about 20% of the interrogated DNA. Sanger sequencing is still used but faces diminishing clinical applicability, as other more testing methods are available for targeted mutation analysis (e.g., allelic discrimination assays, melt curve analysis, and digital droplet PCR, among many others).

In the late 1980s to 1990s, Pål Nyrén and colleagues pioneered the pyrosequencing technique, obtaining a nucleotide sequence in real time through DNA synthesis and enzymatic luminescence [69, 70]. Pyrosequencing preceded "second-generation" sequencing, which is also referred to as next-generation sequencing (NGS) or massively parallel sequencing. Made possible only through technological improvements in microfabrication and high-resolution imaging, NGS has been one of the most disruptive technologic advances in molecular biology in the last few decades. While there are differences between individual technologies, general steps include:

- Library preparation: Sample preparation including nucleic acid fragmentation and ligation of adaptor/barcode nucleotide sequences to the 5′- and 3′-fragment ends. Adapters usually include universal priming sequences for amplification.
- Clonal amplification: Separation of library fragments in three-dimensional space, allowing for amplification/identification of unique clonal PCR products on a solid-phase substrate. Several million to billions of reaction foci are sequenced per run.
- Sequence analysis: Each of the generated sequence reads from the amplification step is bioinformatically processed for removal of low-quality reads, mapping to a reference genome, and identification/annotation of sequence variants.
- Variant interpretation: Identified variants are evaluated for pathogenicity and clinical implications.

No polymerase is perfect, and the clonal amplification step will introduce some amplification-related sequence errors. Depending on the technologic platform, sequencing error rates range from <0.01% to ~1%. There may be software-related decreased sensitivity in the detection of large insertions/deletions, and some assays may have decreased read accuracy in homopolymer tracts. Overall sensitivity for detection of variants is generally better than Sanger sequencing, and the treating physician can interrogate for multiple potential pathogenic mutations in one study. Many "third-generation" sequencing platforms focus on single molecule analysis and may bypass the clonal amplification step required for NGS, but these technologies still have limited clinical application and will not be discussed in further detail.

While NGS is popularly associated with whole genome and whole exome sequencing, many targeted "gene panel" assays are available for clinical oncologic use. There is growing adoption and clinical utility of targeted NGS to identify somatic mutations in lymphoma, and mutation profiling panels for various malignant lymphomas are available. Copy number variations and translocations can be difficult to reliably identify using NGS, but these abnormalities are being successfully included and studied in cancer panels [71, 72]. The technology also has numerous other potential applications in the setting of lymphoma, including evaluation of circulating cell-free DNA, clonality studies, IGH-V analysis, minimal residual disease evaluation, and others.

Immunoglobulin Gene Clonality Studies

B-cells undergo a somatic rearrangement of the immunoglobulin genes as a course of normal development. This physiologic process provides a convenient means to assess clonality status in mature B-cell populations. While histomorphology and supporting immunophenotyping studies are typically sufficient to diagnose follicular or mantle cell lymphomas, molecular clonality studies may sometimes have utility in the workup of suspected lymphoproliferations or to clarify associations between phenotypically dissimilar lymphomas arising in one individual.

The current standard approach uses consensus primer sets for PCR amplification targeting the V-region and J-regions of the immunoglobulin gene, followed by amplicon fragment sizing by electrophoresis. Because each B-lymphocyte normally harbors a unique immunoglobulin gene rearrangement, polyclonal lymphocyte populations will yield multiple sized fragments in a normal/Gaussian distribution pattern on a capillary electropherogram. A variety of different amplicon banding patterns may arise in the setting of clonal or oligoclonal populations, but monoclonal populations are typically acknowledged when one or two dominant peaks are found to be significantly higher than the background peak pattern. Pattern interpretation can be somewhat subjective, but guidelines to assist in the interpretation and reporting of immunoglobulin clonality testing are readily available [73].

Studies targeting the *IGH* and sometimes the *IGK* loci are commonly available tests in clinical molecular laboratories. The limit of detection for this type of analysis is around 25% clonal B-cells in a background of polytypic mononuclear cells. A few other factors may potentially impair the reliability of clonality detection if the *IGH* locus is evaluated only. The primary causes of impairment include imprecise annealing of consensus primers to all potential V and J segments and primer site alterations incurred through somatic hypermutation in germinal center and post-germinal center lymphomas. Evaluation of the *IGH* locus alone has been found to lead to a high false negative rate of up to 30% depending on B-cell lymphoma type [74, 75]. This phenomenon impacts cases of follicular lymphoma much more than cases of mantle cell lymphoma. Theoretically, all mature B-cell malignancies will have undergone rearrangement of *IGK* regardless of functional light chain expression. Thus, a strategy of combined evaluation of the *IGH* V_H-J_H and *IGK* loci has

been shown to have high clonality detection rates of about 98% overall [76]. It must be noted that clonality studies cannot be used to assign lineage to a neoplastic lymphoproliferation. Co-existing clonal T-cell receptor gene rearrangements can be detected in nearly 30% of mature B-cell malignancies, and cross-lineage Ig rearrangements can also be found in a minority of T-cell malignancies [74].

NGS technologies are currently being applied to immunoglobulin gene rearrangements for clonality assessment, and some "clonality by sequencing" assays are clinically available [77–79]. Early comparative studies have shown >95% concordance clonality results with capillary electrophoresis, and discordances appear to be related to higher sensitivity/resolution associated with NGS testing with improved detection in cases with high somatic hypermutation [78]. Having knowledge of the clonal immunoglobulin gene sequence proffers additional potential clinical applications, such as assessment for minimal residual disease, determination of immunoglobulin heavy chain variable region gene (Ig-V_H) usage, or possible tumor detection through liquid biopsy evaluation of circulating tumor DNA.

Molecular Assessment of Classic Translocations

The t(14;18)(q32;q21) *IGH/BCL2* and t(11;14)(q13;q32) *CCND1/IGH* translocations are considered the molecular hallmarks of follicular lymphoma (FL) and mantle cell lymphoma (MCL), respectively. Both of these translocations have been thoroughly characterized at the molecular level. Although breakpoints may be dispersed over large genomic regions, the majority of translocations in the *BCL2* and *CCND1* genes demonstrate regional clustering and may be amenable to PCR detection [80–83].

Most *BCL2* translocation breakpoints associated with follicular lymphoma have been found to localize to the 3′ region of the gene and cluster to three main locations (Fig. 4.2) [84–86]. The major breakpoint region (MBR) accounts for about half of all the translocation breaks and localizes to a ~175 bp area within the 3′ noncoding region of exon 3. The two remaining named cluster regions are found in a ~29 kb region adjacent centromerically to the MBR. The intermediate cluster region (ICR,

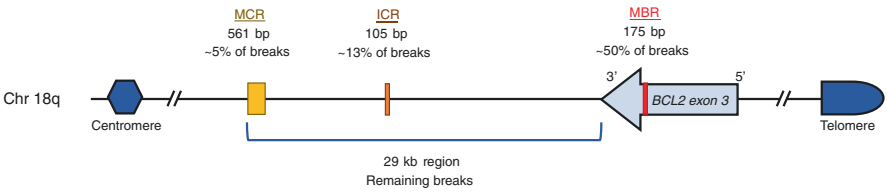


Fig. 4.2 *BCL2* translocation breakpoint cluster map (not drawn to scale). MBR major breakpoint region, ICR intermediate cluster region, MCR minor cluster region. Very rare 5′ *BCL2* breaks have been reported in follicular lymphoma, but are not annotated here

105 bp) and minor cluster region (MCR, 561 bp) account for ~13% and 5% of translocation breaks, respectively. The remaining *BCL2* breakpoints are generally scattered throughout this ~29 kb region. The *IGH* gene breakpoints all fall within J_H. Activation-induced cytidine deaminase (AID) functions physiologically to initiate the processes of class switch recombination and somatic hypermutation, but has been implicated in B-cell translocations. Breaks occur at the DNA sequence motifs CG (CpG) or WGCW (where W = A or T), both of which are known AID targets. Each of these sequence motifs in the described breakage zones is a hotspot for translocation [85].

Unlike the breaks associated with *BCL2* translocations, most breaks associated with *CCND1* rearrangements do not specifically cluster. Instead, most are distributed across a large ~344 kb region adjacent to the *CCND1* gene (Fig. 4.3). A "major translocation cluster" can be found within this region about 110 kb away from the *CCND1* gene. This cluster encompasses an area of ~150 bp and represents roughly 30% of the translocation breaks. The remaining 70% are widely distributed throughout the surrounding ~344 kb territory [84, 87]. For mantle cell lymphoma, the t(11;14) *CCND1* rearrangement pairs to an IGH-J segment. However, in some cases of B-NHL and in multiple myeloma, the translocations have also been found to pair at IGH gamma switch regions [88]. About half of the *CCND1* breaks are found near AID sequence motifs; most are at CG (CpG) motifs, while half of the remaining breaks are near WGCW. Breaks in at the CG motifs are scattered on both sides of the major translocation cluster, whereas WGCW motif breaks tend to occur telomeric to the major translocation cluster [87]. While it may be somewhat surprising that AID targets CpG and WGCW sites so distant from the *CCND1* gene, around 10–20% of AID-dependent translocation hotspots appear intergenic [89]. While PCR may be acceptable for detection of most *BCL2* translocations, it has generally been considered suboptimal for t(11;14) translocations. Designing primers to cover randomly dispersed breaks over hundred kilobases for conventional PCR analysis is generally untenable, but newer methodologies and RNA-based NGS assays are being applied to bridge this gap [90–92].

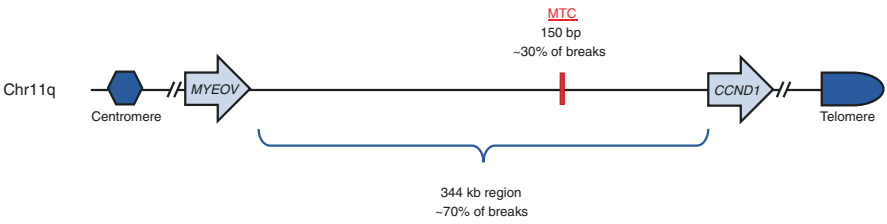


Fig. 4.3 CCND1 translocation breakpoint cluster map (not drawn to scale). MTC major translocation cluster

Minimal Residual Disease Assessment

PCR testing for these fusions is not routinely ordered in the diagnostic workup of either type of lymphoma. However, there has been a significant amount of recent interest in using PCR-based testing for the detection of minimal residual disease (MRD, also referred to as measurable residual disease). MRD assessment is routine and standard of care for certain leukemias, but currently the application in solid tissue-based lymphomas is still fairly novel. Current strategies are aimed at the detection of circulating tumor cells or cell-free circulating tumor DNA (ctDNA). ctDNA is nucleic acid that has been released into the blood by lymphoma cells during necrosis or apoptosis.

Methods applied to the detection of MRD include flow cytometry, PCR and variants like digital droplet PCR (ddPCR), and NGS-based protocols. Flow cytometry-based MRD detection limits range from 10^{-4} to 10^{-5}, whereas real-time quantitative PCR (RQ-PCR) techniques appear to have a limit of 10^{-5} [93–96]. NGS-based MRD assays offer the highest technical sensitivity at 10^{-6}, contingent on DNA input from three million bone marrow cells [97–99]. Flow cytometry and RQ-PCR may be equally suited for MRD assessment in certain disorders such as chronic lymphocytic leukemia, but RQ-PCR and NGS-based analysis have been more broadly applied techniques in the primarily solid tissue-based follicular and mantle cell lymphomas [100]. Furthermore, molecular methods do not require living/viable tissue samples, workflow is more automated, and there is less inter-observer interpretive variation. ddPCR is a newer highly sensitive high-throughput PCR technology that allows for absolute quantification of genetic aberrancies, rather than the relative quantification generated by RQ-PCR. While still not in wide clinical use, this technique has been demonstrated to detect relevant somatic mutations in ctDNA from the blood of patients with diffuse large B-cell lymphoma (DLBCL) at a sensitivity of 5×10^{-4} and is being adapted for general use in mature lymphoproliferative disorders (0.05%) [101, 102].

As mentioned previously, PCR-based assays will detect gene fusion products for both the t(14;18)(q32;q21) and t(11;14) translocations in a significant minority of otherwise healthy individuals [11, 48]. However, studies in follicular lymphoma have found the incidence of non-neoplastic IGH/BCL2 rearrangements following chemotherapy to be low, suggesting that that PCR positivity for t(14;18) in the post-treatment setting has a prognostic role for follow-up care decisions [11]. Empirically, early study results using consensus PCR to detect clonal t(14;18) translocation and/or IGH clonality appear to track with treatment response to therapy and support the use of MRD testing as an early predictor of survival in follicular and mantle cell lymphomas [93, 94, 103–107].

When using RQ-PCR for MRD diagnostics, interrogating for translocation fusion products is technically more straightforward than targeting the *IGHV* gene to detect a persistent clone. The junctional regions for an individual lymphoma clone would need to be identified before an RQ-PCR study could be designed for MRD monitoring, and the degree or ongoing process of somatic hypermutation within a clonal population (as is seen in some follicular lymphomas) could hamper

accurate results [108, 109]. However, many of the technical RQ-PCR challenges are negated by NGS-based methods, where clonality assessment is based upon the actual nucleotide sequence. Immunoglobulin clones are considered malignant if they demonstrate a frequency of >5% among all immunoglobulin sequences derived from the lymphoma specimen, and NGS allows for the tracking of multiple clonal populations. While immunoglobulin sequences are a fairly obvious target choice, any tumor-specific ctDNA can potentially be used for MRD by NGS. In a study of DLBCL, gene mutation panel-directed NGS studies were able to detect ctDNA at the time of radiographic disease progression and before relapse in 73%. Patients with ctDNA detected any time after remission had significantly worse progression-free survival compared to ctDNA-negative patients [110]. MRD assessment looks to be a highly relevant tool for discovering the presence of subclinical disease in lymphoma. Although MRD-guided management protocols have not yet been standardized, multiple options for measurement are available. A summary of strengths and weaknesses for selected MRD testing methods are found in Table 4.1.

Table 4.1 Methods for MRD assessment in lymphoma

	Flow cytometry	RQ-PCR	ddPCR	NGS Ig/clonality	NGS Gene panel
Sample requirements	Fresh/viable cells only	Fresh or FFPE tissue	Fresh or FFPE tissue	Fresh or FFPE tissue	Fresh or FFPE tissue
Detection limit	10^{-4} to 10^{-5}	10^{-5}	10^{-5}	10^{-6}	10^{-6}
Availability	Commercially available, MRD flow not offered by all flow labs	Primarily research setting	Primarily research setting	Commercially available	Primarily research setting
Advantages	More readily available than other methods	Well validated in mantle cell lymphoma	Absolute quantification of genetic lesion Fast	Reagents and commercial kits readily available	Generates extensive genomic information Can track clonal evolution
Disadvantages	Less sensitive Requires circulating tumor cells Standardization between labs is poor	Limited to only a few genetic lesions High level of expertise needed if targeting Ig Time-/labor-intensive	Multiplexing and assessment of multiple lesions may be limited	MRD evaluation requires neoplastic sequence from primary sample	Sensitivity limited by panel size and sequencing depth Limited detection if low allele frequency

Interpretive Considerations

As discussed previously, the t(14;18) and t(11;14) rearrangements are viewed as early aberrancies for the founding long-lived neoplastic clones that will develop into follicular and mantle cell lymphomas. However, a person must subsequently acquire various additional driver abnormalities for the "in situ" lesional cells to become a fully manifested lymphoma. In addition to structural and copy number aberrancies, the molecular profiling has added another layer of complexity to our understanding of the mutational landscape (see Tables 4.2 and 4.3). The means by which these variants drive pathogenesis have been described elsewhere in this book, and the following sections will focus primarily on providing context to some variants as related to disease behavior.

Follicular Lymphoma

Sequence variants in epigenetic regulators are common, and the profile of recurring aberrancies that occur early and late in disease development has been described. Mutations affecting epigenetic regulators are common and are thought to be early drivers of pathogenesis [111–114]. Multiple points in epigenetic regulation are hit, including nucleosome remodelers in the SWI/SNF complex family, linker histone H1 and H2 family genes, methyltransferases, and acetyltransferases. Over 70% of cases will carry concurrent mutations in histone modifying enzymes such as *CREBBP*, *EZH2*, *MEF2B*, and *KMT2D*; nearly a third show mutation of at least one linker histone gene [111]. Genes in pathways in B-cell development and signaling pathways that promote tumor cell survival, such as JAK-STAT and NFκB, are also recurrently affected.

Somatic mutations in *KMT2D*, *CREBBP*, *EZH2*, *STAT6*, and *TNFRSF14* are thought to represent early clonal events ("driver" mutations) and are longitudinally stable across subclones with disease progression [111]. *KMT2D*, *CREBBP*, and *TNFRSF14* often harbor compound heterozygous mutations paired with either deletions or acquired uniparental disomy (aUPD), resulting in biallelic inactivation. EPHA7, EP300, and MEF2B have also been proposed to be early driver genes [25, 115, 116]. Similar to what has been described cytogenetically, molecular characterization of follicular lymphoma shows subclonal evolution of genetically divergent tumor populations with a shared set of founder mutations [29, 39, 111]. Variant allele frequency discordance and their distribution between clones cannot be accurately predicted by assessing clustering of variant frequencies from bulk tumor alone [39].

No single genetic aberrancy acquisition has been found that drives transformation, but a variety of late "progressor" events have been described which may herald the onset of aggressive disease. In general, the genetic landscape of high-grade or aggressive transformation is more complex than the preceding indolent follicular lymphoma, showing higher numbers of missense variants, small insertions and

Table 4.2 Selected genetic abnormalities in follicular lymphoma

Cytogenetics			
	Chr. region	**Frequency (%)**	**Putative relevance**
Translocation	*BCL2* locus	85–90	t(14;18) is a "diagnostic hallmark," few cases may be negative, and there is no prognostic relevance
	BCL6 locus	45	Associated with high-grade histology
	MYC locus	<5	Poor prognosis, translocation associated with transformation
Gains	5, 12, 18q, 19, 20, and 21	–	Shorter survival
	8q24 (*MYC)*		Amplification described as a late event
	X	–	Shorter survival in men
Losses	1p21–22, 6q23–26, or 17p	–	Shorter survival
	1p36.22-p36.33*, 6q21-q24.3	–	Associated with transformation, high proliferation; *1p36 deletion may be favorable if the context of low-grade t(14;18)-negative nodal follicular lymphoma with predominantly diffuse growth pattern
Molecular			
	Affected gene	**Frequency (%)**	**Putative relevance**
Epigenetic	*KMT2D (MLL2)*	85	Early clonal event, often compound heterozygous with deletion or acquired uniparental disomy (aUPD)
	CREBBP	33	Early clonal event, often compound heterozygous with deletion or aUPD
	EP300	10	Possible early event
	EZH2	60	Early clonal event, amplification described as a late event, may have better prognosis vs wt-*EZH2* cases
	MEF2B	15–20	–
	ARID1A	10–15	Good prognostic marker
	HIST1H1C	5	–
	HIST1H1E	15–20	–
Immune modulation	*TNFRSF14*	45–65	Early clonal event, often compound heterozygous with deletion or aUPD
	B2M	10–15	Enriched in transformation
	FAS (TNFRSF6)	5	Enriched in transformation
JAK-STAT	*SOCS1*	5–10	–
	STAT3	<5	–
	STAT6	12	Early clonal event, may show slight enrichment at transformation

(continued)

Table 4.2 (continued)

BCR/NFκB signaling	*CARD11*	11	–
	TNFAIP3	20	Late event, found with transformation
	BCL10	<5	–
	CD79A	<5	–
	CD79B	<5	–
	PRKCB	<5	–
	PLCG2	<5	–
	MYD88	<5	Late event, found with transformation
Transcription factor	*EBF1*	10	Late event, found with transformation
	IKZF3	<5	–
	MEF2B	15	–
B-cell development	*KLHL6*	5	–
Tumor suppressor	*TP53*	<5	Associated with transformation, lost in 17p deletions
	CDKN2A/B	5–10	Worse overall survival, may also be methylated in ~20%
	EPHA7	70	Often inactivating mutations, lost in 6q deletions
IGHV	*V(H)5* subgroup	5–10	Adverse, worse 5-year survival
	V(H)3–48	10	Adverse, increased risk for transformation
	>1 *IGH-V* subgroup usage	<5	Adverse, worse 5-year survival

deletions, copy number changes, and structural rearrangements. The majority of cases with high-grade transformation contain late "progressor"-type mutations showing hallmarks of AID-mediated aberrant somatic hypermutation [25, 117]. This is a mechanism of genetic instability resulting from abnormal activation of a physiologic process that is ordinarily restricted to the germinal center. Prolonged exposure of the early/founding clone to this potentially deleterious germinal center reaction favors the accumulation of lesions.

Amplifications of *EZH2*, *MDM2*, *MYC*, and *REL* are reported to be enriched at transformation [111]. Mutations in the transcription factor *EBF1* and various regulators of the NFκB pathway (such as *MYD88* and *TNFAIP3*) have also been found as late events associated with transformation [111]. Increased NFκB pathway activation is phenotypically reflected by *IFR4* (*MUM1*) expression, which may explain why *IRF4* expression has separately been associated with shortened progression-free survival [34, 118, 119]. Other secondary alterations associated with transformation and poor outcome include activating alterations of oncogenes such as *MYC* and *CCND3* and loss or inactivation of tumor suppressor genes such as *TP53*, *CDKN2A/B*, and *B2M* [25, 34, 120].

NOTCH1 and NOTCH2 mutations are relatively common in splenic marginal zone lymphoma and can be found in other lymphoid neoplasms, but are found in only ~5% of follicular lymphoma. However, when present in the setting of follicular

Table 4.3 Selected genetic abnormalities in mantle cell lymphoma

Cytogenetics			
	Chr. region	**Frequency (%)**	**Putative relevance**
Translocation	*CCND1* locus	>95	t(11;14) is a "diagnostic hallmark," rare cases may be negative, and there is no prognostic relevance
	MYC locus		Prognostically adverse
	CCND2, *CCND3*	Rare	May be found in rare t(11;14)-negative cases, no prognostic relevance
Gains	3q, 12q	–	Prognostically adverse
	"Tetraploidy"	–	Associated with blastoid/pleomorphic variants
Losses	9p (*CDKN2A/B*), 9q21–q32, 17p (*TP53*)	–	Prognostically adverse, blastoid/pleomorphic variants have higher frequency of 9p and 17p loss
Molecular			
	Affected gene	**Frequency (%)**	**Putative relevance**
Cell cycle	*CCND1*	18–35	May decrease response to ibrutinib, associated with higher proliferation and worse outcomes
Epigenetic	*KMT2C* (*MLL3*)	~16	–
	KMT2D (*MLL2*)	10–20	–
	NSD2 (*WHSC1*)	5–15	Possible correlation with shorter survival
NFκB signaling	*TRAF2*	<10	–
	BIRC3	5–10	May decrease response to ibrutinib
	CARD11	5	May decrease response to ibrutinib and lenalidomide
Development/homeostasis	*NOTCH1*	5–15	More aggressive behavior, poor survival
	NOTCH2	~5	More aggressive behavior, poor survival
Ubiquitination proteasome	*UBR5*	5–20	–
Tumor suppressor	*TP53*	10–30	Prognostically adverse, higher frequency in blastoid/pleomorphic variants
	CDKN2A/B	Loss, 18–31 Sequence mut, <10	Prognostically adverse, higher frequency in blastoid/pleomorphic variants
	ATM (11q22-q23)	Loss or sequence mut, 20–60	Associated with complex karyotypes, no univariate correlation with tumor behavior/outcomes

(continued)

Table 4.3 (continued)

IGHV	V(H)3–21	10–19	Associated with longer median survival
miRNA	miR-18a, miR-18b*, miR-20b, miR-363	–	Individually associated with high proliferation group, *miR-18b associated with resistance to chemotherapy and poor outcomes
	miR-190, miR-149	–	High expression individually associated with shorter survival
	miR-149, miR-34a, miR-649, miR-483-5p, miR-565	–	Low expression individually associated with shorter survival

lymphoma, cases are reported to have distinctive clinical and pathologic features including female predominance, frequent splenic involvement, lower frequency of t(14;18) at ~14%, and more frequent presence of a component of diffuse large B-cell lymphoma (57%) [121].

With increased NGS-based evaluation of immunoglobulins for clonality and other applications, lymphoma immunoglobulin heavy chain variable (*IGHV*) gene usage and mutational status may become more readily available for clinical use. The usage of specific V(H) segments is heavily biased in follicular lymphoma. From the six *IGHV* subgroups, subgroup V(H)3 is utilized most frequently, representing about 50–60% of all cases [122]. Highly used individual sequences include V(H)3-23 (~15%), V(H)4-34 (~15%), V(H)3-48 (~10%), V(H)3-30 (5–10%), V(H)3-21 (5–10%), and V(H)3-15 (5–10%) [123]. Usage of the V(H)5 subgroup or cases with *IGH-V* sequences from more than one subgroup appear to have a less favorable prognosis, decreasing the estimated 5-year survival to about 50–60%, when compared to a 5-year survival of 95% found with other *IGHV* subgroups [122]. Additionally, usage of V(H)3-48 may predict for high-grade transformation [123]. About 15% of cases will have unmutated *IGHV* status (>98% homology), and in contrast to chronic lymphocytic leukemia (CLL), unmutated *IGHV* genes and utilization of V(H)3-21 do not appear to have an adverse prognosis [122].

Following the clarification of the molecular landscape of follicular lymphoma, newer genetic prognostic indexes have been developed, such as the m7-FLIPI [124]. This risk model combines the follicular lymphoma international prognostic index (FLIPI), Eastern Cooperative Oncology Group (ECOG) performance status, and the mutation status of seven genes: *EZH2*, *ARID1A*, *MEF2B*, *EP300*, *FOXO1*, *CREBBP*, and *CARD11*. This system outperformed the FLIPI, both alone and when combined with the ECOG performance status. The improved risk stratification for failure-free survival was achieved mostly through reclassifying a subset of high-risk FLIPI patients into a low-risk m7-FLIPI risk group. Unfortunately, m7-FLIPI case cohort was insufficient to adequately stratify for risk of transformation.

Comparative gene expression profile studies against de novo diffuse large B-cell lymphoma indicate that transformed follicular lymphoma more closely resembles germinal center-type diffuse large B-cell lymphoma (GCB-DLBCL) than activated

B-cell type (ABC-DLBCL) [25]. Unique combinations of genetic lesions that are found in transformed follicular lymphoma, but are not observed or extremely rare in de novo GCB-DLBCL, include biallelic loss of *CDKN2A/B* and mutations of *STAT6*, *ARID1A*, and *FAS* [25]. Aberrancies of *KMT2D* (*MLL2*), *CREBBP*, *BCL2* translocations, and other "early" clonal events in follicular lymphoma are found in both cases of transformed lymphoma and de novo DLBCL, but are significantly enriched in cases derived from follicular lymphoma transformation [25]. While this finding is not unexpected, it does also suggest that a subset of GCB-DLBCL may arise from the previously discussed early/founding long-lived neoplastic clone.

Mantle Cell Lymphoma

The t(11;14) translocation is generally recognized the initiating step in founding a mantle cell lymphoma clone, but secondary genetic lesions appear to drive oncogenesis and likely influence clinical behavior. Two major subgroups are recognized by the 2016 WHO classification system: classic nodal disease which typically has an aggressive course and leukemic non-nodal disease which is associated with indolent course and good outcomes. However, patients with classic mantle cell lymphoma may still present with extremely varied clinical presentations. The list of genes affected by recurrent somatic sequence variants is shorter than what is reported generally reported for follicular lymphoma, but a variety of impacted functional groups are represented, including cell cycle regulators, epigenetic regulators, NFκB signaling, development/homeostasis, ubiquitination proteasome, and tumor suppressors (among others) [125–132]. Inactivating mutations of the *ATM* gene at 11q22-23 are the most frequently identified variants, found in 40–50% of cases. Other genes that are generally affected at frequencies >10% include *CCND1*, *KMT2C* (*MLL3*), *KMT2D* (*MLL2*), *NSD2* (*WHSC1*), *NOTCH1*, and *TP53*.

The *ATM* is a tumor suppressor gene that encodes a protein involved in signaling DNA damage. Inactivating missense and truncating variants in this gene are the most frequent secondary mutations in mantle cell lymphoma, but some reports have found no associations to clinical behavior or outcomes on univariate analysis [127, 132]. In contrast, cases that acquire loss or inactivating variants of *TP53* have a significantly shortened median survival relative to cases with wild-type alleles [127, 133]. Cases with *ATM* abnormalities have defects in double-strand DNA break repair, have impaired apoptosis, and are associated with increased karyotype complexity [134, 135]. *ATM* also mediates some p53-dependent apoptosis pathways. Although there is no strong selective advantage to acquire co-mutation of both *ATM* and *TP53* abnormalities, various combination of missense and deletions of these two genes confer distinctly gene expression profiles that differ from wild-type cases, the consequences of which are yet undetermined [127]. Cells with non-functional *ATM* are also known to show increased radiosensitivity, and data from patients with mantle cell lymphoma indicate that radiation is an effective treatment strategy even in heavily pretreated and chemorefractory patients [136, 137].

Assessment of the tumor proliferation rate with Ki-67 immunohistochemistry is presently more critical than assessment of molecular markers for prognostic evaluation, and no molecular markers are incorporated into the commonly utilized mantle cell lymphoma international prognostic index (MIPI) [138, 139]. The incorporation of molecular genetic lesions to influence treatment decisions has not yet entered routine clinical practice. Regarding sequence aberrancies, only losses of *CDKN2A* and *TP53* appear to have significant negative prognostic impact [140, 141]. Other factors that are putatively linked to inferior outcomes include *MYC* overexpression, *NOTCH1* and/or *NOTCH2* mutations, *NSD2* (*WHSC1*) mutations, and *CCND1* mutations.

NOTCH1 and *NOTCH2* variants have been associated with aggressive tumor behavior and worse overall survival [126, 130]. The activating truncating or frameshifting indel variants cluster in the *NOTCH1* PEST domain, resulting in a prolongation of the encoded protein's half-life and transcriptional activity through interference with *FBXW7* (FBW7)-mediated ubiquitination and degradation of the *NOTCH1* intracellular domain (NICD1) [130, 142, 143]. At least one study has found correlation with shorter survival in patients with NSD2 mutation [144].

The NFκB pathway appears to be constitutively activated in at least a subset of mantle cell lymphoma, and ex vivo treatment with an NFκB inhibitor resulted in cell cycle arrest and apoptosis [145]. Mantle cell lymphoma models that show sensitivity to B-cell signaling inhibitors ibrutinib and sotrastaurin show classic NFκB activation through the B-cell receptor (BCR) signaling pathway. A subset of insensitive cases have been shown to have NFκB activation through an alternative pathway involving *MAP3K14* (*NIK*) in vitro and in vivo [129]. *BIRC3* aberrations are thought to decrease response to ibrutinib by failing to suppress this alternate *MAP3K14*-based NFkB pathway, and this has been proposed as a therapeutic target in BIRC3-mutated lymphoma. Mutations in *CARD11*, a scaffold protein required for BCR-induced NFκB activation, have also been found to confer resistance to ibrutinib and lenalidomide [146]. Better understanding of drivers in these molecular pathways may provide more insights for clinical care.

In addition to being a key player in the hallmark t(11;14) translocation, the *CCND1* gene contains a hotspot for recurring mutations in the 3′ untranslated region of exon 1. The variants are thought to arise through somatic hypermutation, produce a truncated mRNA with a longer half-life, and result in increased protein levels [147]. Some of the variants also show a stabilized CCND1 protein through attenuation of threonine-286 phosphorylation, which is important for ubiquitin-proteasome proteolysis [148]. The consequence to tumor behavior appears to be increased proliferation rates, increased resistance to ibrutinib therapy, and shorter overall survival [147–149].

Compatible with its pre-germinal center "naïve" B-cell origin, most mantle cell lymphomas have no or very few somatic mutations in their *IGHV* gene sequences. However, a significant minority of cases (15–40%) will show evidence of somatic hypermutation, indicating that some tumors originate in cells that have been exposed to germinal center mutational processes [150–152]. Unlike CLL, *IGHV* mutation status does not correlate with outcomes in mantle cell lymphoma [152–154]. The study by Kienle et al. did find over-representation of trisomies +3q, +8q, and tetraploidy in the *IGHV*-unmutated group and +12q in the *IGHV*-mutated group [152].

There is biased usage of specific V(H) segments, with V(H)3-21 (10–19%), V(H)4-34 (11–17%), V(H)3-23 (9%), and V(H)4-59 (9%) [153, 154]. Usage of V(H)3-21 tends to show association with an unmutated status, and cases had a significantly longer median survival [150, 152–154].

Although, in most cases, *IGHV* mutation status has no correlation to disease characteristics in mantle cell lymphoma, the uncommon indolent cases with a nonnodal leukemic presentation have been associated with hypermutated *IGHV* and a noncomplex karyotype [55, 150, 155]. This indolent leukemic variant shows 8p21.3 deletion and gain of 8q24.1 loci more often than conventional nodal cases [55]. The study by Fernàndez et al. also identified a 13-gene signature (*RNGTT*, *HDGFRP3*, *FARP1*, *CSNK1E*, *SETMAR*, *HMGB3*, *LGALS3BP*, *PON2*, *CDK2AP1*, *DBN1*, *CNR1*, *CNN3*, and *SOX11*) that was highly expressed in conventional disease, but was significantly under-expressed in cases of indolent mantle cell lymphoma [155].

RNA-based gene expression profiling has also been used to more precisely measure tumor proliferation, and this gene expression-based model was able to accurately stratify patient groups relative to survival [49]. Ki-67 evaluation has been used as a surrogate measure, but recent technological improvements have made RNA-based studies of this type more feasible in formalin-fixed paraffin-embedded tissues. Early studies have shown promising improvements to overall survival prognostication, independent of the MIPI [156].

MicroRNAs (miRNAs) are a class of noncoding RNAs that influence the regulation of gene expression. These usually function to silence gene expression by binding to target sites within the 3′ untranslated region on mRNA that immediately follows the translation termination codon, suppressing synthesis and/or initiating mRNA degradation. Deregulation of miRNA has been implicated in mantle cell lymphoma oncogenesis, and certain miRNA profiles have been associated with a high proliferation gene signature [157]. Two larger mantle cell lymphoma studies had some discordance in described miRNA expression profiling, which may be related to differences in miRNA modulation between the peripheral blood and lymph node microenvironment [157–159]. However, both studies found that miRNA profiles altered gene expression in a way that was prognostically meaningful. High expression of miR-18b has been singled out as a marker of poor chemotherapy resistance and poor outcomes [157, 160]. Well-vetted miRNA profiles that are clinically applicable for prognostication and treatment stratification are not yet available, but this exciting line of research appears to hold promise for clinical care.

References

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J. WHO classification of tumours of haematopoietic and lymphoid tissues, Revised 4th ed. Lyon: International Agency for Research on Cancer; 2017.
2. Cooley LD, Lebo M, Li MM, Slovak ML, Wolf DJ. Working Group of the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee. Genet Med. 2013;15(6):484–94.

3. Smits AJ, Kummer JA, de Bruin PC, Bol M, van den Tweel JG, Seldenrijk KA, et al. The estimation of tumor cell percentage for molecular testing by pathologists is not accurate. Mod Pathol. 2014;27(2):168–74.
4. O'Keefe C, McDevitt MA, Maciejewski P. Copy neutral loss of heterozygosity: a novel chromosomal lesion in myeloid malignancies. Blood. 2010;115(14):2731–9.
5. Simons A, Sikkema-Raddatz B, de Leeuw N, Konrad NC, Hastings RJ, Schoumans J. Genome-wide arrays in routine diagnostic of hematological malignancies. Hum Mutat. 2012;33(6):941–8.
6. Etebari M, Navari M, Piccaluga PP. SNPs array karyotyping in non-Hodgkin lymphoma. Microarrays (Basel). 2015;4(4):551–69.
7. Caspersson T, Farber S, Foley GE, Kudynowski J, Modest EJ, Simonsson E, et al. Chemical differentiation along metaphase chromosomes. Exp Cell Res. 1968;49(1):219–22.
8. Fukuhara S, Rowley JD, Variakojis D, Golomb HM. Chromosome abnormalities in poorly differentiated lymphocytic lymphoma. Cancer Res. 1979;39(8):3119–28.
9. Yunis JJ, Oken MM, Kaplan ME, Ensrud KM, Howe RR, Theologides A. Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin's lymphoma. N Engl J Med. 1982;307(20):1231–6.
10. Tilly H, Rossi A, Stamatoullas A, Lenormand B, Bigorgne C, Kunlin A, et al. Prognostic value of chromosomal abnormalities in follicular lymphoma. Blood. 1994;84(4):1043–9.
11. Ladetto M, Drandi D, Compagno M, Astolfi M, Volpato F, Voena C, et al. PCR-detectable nonneoplastic Bcl-2/IgH rearrangements are common in normal subjects and cancer patients at diagnosis but rare in subjects treated with chemotherapy. J Clin Oncol. 2003;21(7):1398–403.
12. Dyer MJ, Zani VJ, Lu WZ, O'Byrne A, Mould S, Chapman R, et al. BCL2 translocations in leukemias of mature B cells. Blood. 1994;83(12):3682–8.
13. Ott G, Katzenberger T, Lohr A, Kindelberger S, Rüdiger T, Wilhelm M, et al. Cytomorphologic, immunohistochemical, and cytogenetic profiles of follicular lymphoma: 2 types of follicular lymphoma grade 3. Blood. 2002;99(10):3806–12.
14. Damaj G, Verkarre V, Delmer A, Solal-Celigny P, Yakoub-Agha I, Cellier C, et al. Primary follicular lymphoma of the gastrointestinal tract: a study of 25 cases and a literature review. Ann Oncol. 2003;14(4):623–9.
15. Oschlies I, Salaverria I, Mahn F, Meinhardt A, Zimmermann M, Woessmann W, et al. Pediatric follicular lymphoma – a clinico-pathological study of a population-based series of patients treated within the No-Hodgkin's Lymphoma--Berlin-Frankfurt-Munster (NHL-BFM) multicenter trials. Haematologica. 2010;95(2):253–9.
16. Goodlad JR, MacPherson S, Jackson R, Batstone P, White J, Scotland and Newcastle Lymphoma Group. Extranodal follicular lymphoma: a clinicopathological and genetic analysis of 15 cases arising at non-cutaneous extranodal sites. Histopathology. 2004;44(3):268–76.
17. Horsman DE, Gascoyne RD, Coupland RW, Coldman AJ, Adomat SA. Comparison of cytogenetic analysis, southern analysis, and polymerase chain reaction for the detection of t(14;18) in follicular lymphoma. Am J Clin Pathol. 1995;103(4):472–8.
18. Baró C, Espinet B, Salido M, García M, Sánchez B, Florensa L, et al. Cryptic IGH/BCL2 rearrangements with variant FISH patterns in follicular lymphoma. Leuk Res. 2011;35(2):256–9.
19. Adachi M, Cossman J, Longo D, Croce CM, Tsujimoto Y. Variant translocation of the bcl-2 gene to immunoglobulin lambda light chain gene in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A. 1989;86(8):2771–4.
20. Yonetani N, Ueda C, Akasaka T, Nishikori M, Uchiyama T, Ohno H. Heterogeneous breakpoints on the immunoglobulin genes are involved in fusion with the 5′ region of BCL2 in B-cell tumors. Jpn J Cancer Res. 2001;92(9):933–40.
21. Katzenberger T, Ott G, Klein T, Kalla J, Müller-Hermelink HK, Ott MM. Cytogenetic alterations affecting BCL6 are predominantly found in follicular lymphomas grade 3B with a diffuse large B-cell component. Am J Pathol. 2004;165(2):481–90.
22. Salaverria I, Siebert R. Follicular lymphoma grade 3B. Best Pract Res Clin Haematol. 2011;24(2):111–9.

23. Díaz-Alderete A, Doval A, Camacho F, Verde L, Sabin P, Arranz-Sáez R, et al. Frequency of BCL2 and BCL6 translocations in follicular lymphoma: relation with histological and clinical features. Leuk Lymphoma. 2008;49(1):95–101.
24. Au WY, Horsman DE, Gascoyne RD, Viswanatha DS, Klasa RJ, Connors JM. The spectrum of lymphoma with 8q24 aberrations: a clinical, pathological and cytogenetic study of 87 consecutive cases. Leuk Lymphoma. 2004;45(3):519–28.
25. Pasqualucci L, Khiabanian H, Fangazio M, Vasishtha M, Messina M, Holmes AB, et al. Genetics of follicular lymphoma transformation. Cell Rep. 2014;6(1):130–40.
26. Callanan MB, Le Baccon P, Mossuz P, Duley S, Bastard C, Hamoudi R, et al. The IgG Fc receptor, FcgammaRIIB, is a target for deregulation by chromosomal translocation in malignant lymphoma. Proc Natl Acad Sci U S A. 2000;97(1):309–14.
27. Schmidt J, Salaverria I, Haake A, Bonzheim I, Adam P, Montes-Moreno S, et al. Increasing genomic and epigenomic complexity in the clonal evolution from in situ to manifest t(14;18)-positive follicular lymphoma. Leukemia. 2014;28(5):1103–12.
28. Johnson NA, Al-Tourah A, Brown CJ, Connors JM, Gascoyne RD, Horsman DE. Prognostic significance of secondary cytogenetic alterations in follicular lymphomas. Genes Chromosomes Cancer. 2008;47(12):1038–48.
29. d'Amore F, Chan E, Iqbal J, Geng H, Young K, Xiao L, et al. Clonal evolution in t(14;18)-positive follicular lymphoma, evidence for multiple common pathways, and frequent parallel clonal evolution. Clin Cancer Res. 2008;14(22):7180–7.
30. Offit K, Parsa NZ, Gaidano G, Filippa DA, Louie D, Pan D, et al. 6q deletions define distinct clinico-pathologic subsets of non-Hodgkin's lymphoma. Blood. 1993;82(7):2157–62.
31. Höglund M, Sehn L, Connors JM, Gascoyne RD, Siebert R, Säll T, Mitelman F, et al. Identification of cytogenetic subgroups and karyotypic pathways of clonal evolution in follicular lymphomas. Genes Chromosomes Cancer. 2004;39(3):195–204.
32. Lestou VS, Gascoyne RD, Sehn L, Ludkovski O, Chhanabhai M, Klasa RJ, et al. Multicolour fluorescence in situ hybridization analysis of t(14;18)-positive follicular lymphoma and correlation with gene expression data and clinical outcome. Br J Haematol. 2003;122(5):745–59.
33. Cheung KJ, Shah SP, Steidl C, Johnson N, Relander T, Telenius A, et al. Genome-wide profiling of follicular lymphoma by array comparative genomic hybridization reveals prognostically significant DNA copy number imbalances. Blood. 2009;113(1):137–48.
34. Kridel R, Sehn LH, Gascoyne RD. Can histologic transformation of follicular lymphoma be predicted and prevented? Blood. 2017;130(3):258–66.
35. Ross CW, Ouillette PD, Saddler CM, Shedden KA, Malek SN. Comprehensive analysis of copy number and allele status identifies multiple chromosome defects underlying follicular lymphoma pathogenesis. Clin Cancer Res. 2007;13(16):4777–85.
36. Cheung KJ, Telenius A, Lai B, Johnson N, Relander T, Steidl C, et al. High frequency of 1p36.32 deletion or loss of heterozygosity in follicular lymphoma (FL). Blood. 2007;110:183.
37. Davies AJ, Rosenwald A, Wright G, Lee A, Last KW, Weisenburger DD, et al. Transformation of follicular lymphoma to diffuse large B-cell lymphoma proceeds by distinct oncogenic mechanisms. Br J Haematol. 2007;136(2):286–93.
38. Lossos IS, Alizadeh AA, Diehn M, Warnke R, Thorstenson Y, Oefner PJ, et al. Transformation of follicular lymphoma to diffuse large-cell lymphoma: alternative patterns with increased or decreased expression of c-myc and its regulated genes. Proc Natl Acad Sci U S A. 2002;99(13):8886–91.
39. Green MR, Gentles AJ, Nair RV, Irish JM, Kihira S, Liu CL, et al. Hierarchy in somatic mutations arising during genomic evolution and progression of follicular lymphoma. Blood. 2013;121(9):1604–11.
40. Katzenberger T, Kalla J, Leich E, Stöcklein H, Hartmann E, Barnickel S, et al. A distinctive subtype of t(14;18)-negative nodal follicular non-Hodgkin lymphoma characterized by a predominantly diffuse growth pattern and deletions in the chromosomal region 1p36. Blood. 2009;113(5):1053–61.

41. Siddiqi IN, Friedman J, Barry-Holson KQ, Ma C, Thodima V, Kang I, et al. Characterization of a variant of t(14;18) negative nodal diffuse follicular lymphoma with CD23 expression, 1p36/TNFRSF14 abnormalities, and STAT6 mutations. Mod Pathol. 2016;29(6):570–81.
42. Gángó A, Bátai B, Varga M, Kapczár D, Papp G, Marschalkó M, et al. Concomitant 1p36 deletion and TNFRSF14 mutations in primary cutaneous follicle center lymphoma frequently expressing high levels of EZH2 protein. Virchows Arch. 2018;473(4):453–62.
43. Henderson LJ, Okamoto I, Lestou VS, Ludkovski O, Robichaud M, Chhanabhai M, et al. Delineation of a minimal region of deletion at 6q16.3 in follicular lymphoma and construction of a bacterial artificial chromosome contig spanning a 6-megabase region of 6q16-q21. Genes Chromosomes Cancer. 2004;40(1):60–5.
44. Van Den Berghe H, Parloir C, David G, Michaux JL, Sokal G. A new characteristic karyotypic anomaly in lymphoproliferative disorders. Cancer. 1979;44(1):188–95.
45. Raynaud SD, Bekri S, Leroux D, Grosgeorge J, Klein B, Bastard C, et al. Expanded range of 11q13 breakpoints with differing patterns of cyclin D1 expression in B-cell malignancies. Genes Chromosomes Cancer. 1993;8(2):80–7.
46. Gladden AB, Woolery R, Aggarwal P, Wasik MA, Diehl JA. Expression of constitutively nuclear cyclin D1 in murine lymphocytes induces B-cell lymphoma. Oncogene. 2006;25(7):998–1007.
47. Lovec H, Grzeschiczek A, Kowalski MB, Möröy T. Cyclin D1/bcl-1 cooperates with myc genes in the generation of B-cell lymphoma in transgenic mice. EMBO J. 1994;13(15):3487–95.
48. Hirt C, Schüler F, Dölken L, Schmidt CA, Dölken G. Low prevalence of circulating t(11;14)(q13;q32)-positive cells in the peripheral blood of healthy individuals as detected by real-time quantitative PCR. Blood. 2004;104(3):904–5.
49. Rosenwald A, Wright G, Wiestner A, Chan WC, Connors JM, Campo E, et al. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. Cancer Cell. 2003;3(2):185–97.
50. Fu K, Weisenburger DD, Greiner TC, Dave S, Wright G, Rosenwald A, et al. Cyclin D1-negative mantle cell lymphoma: a clinicopathologic study based on gene expression profiling. Blood. 2005;106(13):4315–21.
51. Salaverria I, Zettl A, Beà S, Moreno V, Valls J, Hartmann E, et al. Specific secondary genetic alterations in mantle cell lymphoma provide prognostic information independent of the gene expression-based proliferation signature. J Clin Oncol. 2007;25(10):1216–22.
52. Gesk S, Klapper W, Martín-Subero JI, Nagel I, Harder L, Fu K, et al. A chromosomal translocation in cyclin D1-negative/cyclin D2-positive mantle cell lymphoma fuses the CCND2 gene to the IGK locus. Blood. 2006;108(3):1109–10.
53. Espinet B, Salaverria I, Beà S, Ruiz-Xivillé N, Balagué O, Salido M, et al. Incidence and prognostic impact of secondary cytogenetic aberrations in a series of 145 patients with mantle cell lymphoma. Genes Chromosomes Cancer. 2010;49(5):439–51.
54. Beà S, Ribas M, Hernández JM, Bosch F, Pinyol M, Hernández L, et al. Increased number of chromosomal imbalances and high-level DNA amplifications in mantle cell lymphoma are associated with blastoid variants. Blood. 1999;93(12):4365–74.
55. Rubio-Moscardo F, Climent J, Siebert R, Piris MA, Martín-Subero JI, Nieländer I, et al. Mantle-cell lymphoma genotypes identified with CGH to BAC microarrays define a leukemic subgroup of disease and predict patient outcome. Blood. 2005;105(11):4445–54.
56. Beà S, Salaverria I, Armengol L, Pinyol M, Fernández V, Hartmann EM, et al. Uniparental disomies, homozygous deletions, amplifications, and target genes in mantle cell lymphoma revealed by integrative high-resolution whole-genome profiling. Blood. 2009;113(13):3059–69.
57. Kohlhammer H, Schwaenen C, Wessendorf S, Holzmann K, Kestler HA, Kienle D, et al. Genomic DNA-chip hybridization in t(11;14)-positive mantle cell lymphomas shows a high frequency of aberrations and allows a refined characterization of consensus regions. Blood. 2004;104(3):795–801.
58. Royo C, Salaverria I, Hartmann EM, Rosenwald A, Campo E, Beà S. The complex landscape of genetic alterations in mantle cell lymphoma. Semin Cancer Biol. 2011;21(5):322–34.

59. Camacho FI, García JF, Cigudosa JC, Mollejo M, Algara P, Ruíz-Ballesteros E, et al. Aberrant Bcl6 protein expression in mantle cell lymphoma. Am J Surg Pathol. 2004;28(8):1051–6.
60. Vaishampayan UN, Mohamed AN, Dugan MC, Bloom RE, Palutke M. Blastic mantle cell lymphoma associated with Burkitt-type translocation and hypodiploidy. Br J Haematol. 2001;115(1):66–8.
61. Ott G, Kalla J, Ott MM, Schryen B, Katzenberger T, Müller JG, et al. Blastoid variants of mantle cell lymphoma: frequent bcl-1 rearrangements at the major translocation cluster region and tetraploid chromosome clones. Blood. 1997;89(4):1421–9.
62. Parry-Jones N, Matutes E, Morilla R, Brito-Babapulle V, Wotherspoon A, Swansbury GJ, et al. Cytogenetic abnormalities additional to t(11;14) correlate with clinical features in leukaemic presentation of mantle cell lymphoma, and may influence prognosis: a study of 60 cases by FISH. Br J Haematol. 2007;137(2):117–24.
63. Ferrer A, Salaverria I, Bosch F, Villamor N, Rozman M, Beà S, et al. Leukemic involvement is a common feature in mantle cell lymphoma. Cancer. 2007;109(12):2473–80.
64. Heyer EE, Deveson IW, Wooi D, Selinger CI, Lyons RJ, Hayes VM, et al. Diagnosis of fusion genes using targeted RNA sequencing. Nat Commun. 2019;10(1):1388.
65. Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. Am J Pathol. 2002;161(6):1961–71.
66. Wickham CL, Sarsfield P, Joyner MV, Jones DB, Ellard S, Wilkins B. Formic acid decalcification of bone marrow trephines degrades DNA: alternative use of EDTA allows the amplification and sequencing of relatively long PCR products. Mol Pathol. 2000;53(6):336.
67. Reineke T, Jenni B, Abdou MT, Frigerio S, Zubler P, Moch H, et al. Ultrasonic decalcification offers new perspectives for rapid FISH, DNA, and RT-PCR analysis in bone marrow trephines. Am J Surg Pathol. 2006;30(7):892–6.
68. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A. 1977;74(12):5463–7.
69. Nyrén P. Enzymatic method for continuous monitoring of DNA polymerase activity. Anal Biochem. 1987;167(2):235–8.
70. Ronaghi M, Karamohamed S, Pettersson B, Uhlén M, Nyrén P. Real-time DNA sequencing using detection of pyrophosphate release. Anal Biochem. 1996;242(1):84–9.
71. Kluk MJ, Lindsley RC, Aster JC, Lindeman NI, Szeto D, Hall D, et al. Validation and implementation of a custom next-generation sequencing clinical assay for hematologic malignancies. J Mol Diagn. 2016;18(4):507–15.
72. Duncavage EJ, Abel HJ, Szankasi P, Kelley TW, Pfeifer JD. Targeted next generation sequencing of clinically significant gene mutations and translocations in leukemia. Mod Pathol. 2012;25(6):795–804.
73. Langerak AW, Groenen PJ, Brüggemann M, Beldjord K, Bellan C, Bonello L, et al. EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. Leukemia. 2012;26(10):2159–71.
74. Diss TC, Pan L. Polymerase chain reaction in the assessment of lymphomas. Cancer Surv. 1997;30:21–44.
75. Wan JH, Trainor KJ, Brisco MJ, Morley AA. Monoclonality in B cell lymphoma detected in paraffin wax embedded sections using the polymerase chain reaction. J Clin Pathol. 1990;43(11):888–90.
76. Evans PA, Pott C, Groenen PJ, Salles G, Davi F, Berger F, et al. Significantly improved PCR-based clonality testing in B-cell malignancies by use of multiple immunoglobulin gene targets. Report of the BIOMED-2 Concerted Action BHM4-CT98-3936. Leukemia. 2007;21(2):207–14.
77. Scheijen B, Meijers RWJ, Rijntjes J, van der Klift MY, Möbs M, Steinhilber J, et al. Next-generation sequencing of immunoglobulin gene rearrangements for clonality assessment: a technical feasibility study by EuroClonality-NGS. Leukemia. 2019;33(9):2227–40.
78. Arcila ME, Yu W, Syed M, Kim H, Maciag L, Yao J, et al. Establishment of immunoglobulin heavy (IGH) chain clonality testing by next-generation sequencing for routine characterization of B-cell and plasma cell neoplasms. J Mol Diagn. 2019;21(2):330–42.

79. Monter A, Nomdedéu JF. ClonoSEQ assay for the detection of lymphoid malignancies. Expert Rev Mol Diagn. 2019;19(7):571–8.
80. Kneba M, Eick S, Herbst H, Willigeroth S, Pott C, Bolz I, et al. Frequency and structure of t(14;18) major breakpoint regions in non-Hodgkin's lymphomas typed according to the Kiel classification: analysis by direct DNA sequencing. Cancer Res. 1991;51:3243–50.
81. Ngan BY, Nourse J, Cleary ML. Detection of chromosomal translocation t(14;18) within the minor cluster region of bcl-2 by polymerase chain reaction and direct genomic sequencing of the enzymatically amplified DNA in follicular lymphomas. Blood. 1989;73:1759–62.
82. Pott C, Tiemann M, Linke B, Ott MM, von Hofen M, Bolz I, et al. Structure of Bcl-1 and IgH-CDR3 rearrangements as clonal markers in mantle cell lymphomas. Leukemia. 1998;12:1630–7.
83. Williams ME, Swerdlow SH, Meeker TC. Chromosome t(11;14)(q13;q32) breakpoints in centrocytic lymphoma are highly localized at the bcl-1 major translocation cluster. Leukemia. 1993;7:1437–40.
84. Willis TG, Dyer MJS. The role of immunoglobulin translocations in the pathogenesis of B-cell malignancies. Blood. 2000;96:808–22.
85. Lieber MR. Mechanisms of human lymphoid chromosomal translocations. Nat Rev Cancer. 2016;16:387–98.
86. Weinberg OK, Ai WZ, Mariappan MR, Shum C, Levy R, Arber DA. “Minor” BCL2 breakpoints in follicular lymphoma: frequency and correlation with grade and disease presentation in 236 cases. J Mol Diagn. 2007;9(4):530–7.
87. Greisman HA, Lu Z, Tsai AG, Greiner TC, Yi HS, Lieber MR. IgH partner breakpoint sequences provide evidence that AID initiates t(11;14) and t(8;14) chromosomal breaks in mantle cell and Burkitt lymphomas. Blood. 2012;120(14):2864–7.
88. Chesi M, Bergsagel PL, Brents LA, Smith CM, Gerhard DS, Kuehl WM. Dysregulation of cyclin D1 by translocation into an IgH gamma switch region in two multiple myeloma cell lines. Blood. 1996;88:674–81.
89. Klein IA, Resch W, Jankovic M, Oliveira T, Yamane A, Nakahashi H, et al. Translocation-capture sequencing reveals the extent and nature of chromosomal rearrangements in B lymphocytes. Cell. 2011;147(1):95–106.
90. Kim B, Lee H, Shin S, Lee ST, Choi JR. Clinical evaluation of massively parallel RNA sequencing for detecting recurrent gene fusions in hematologic malignancies. J Mol Diagn. 2019;21(1):163–70.
91. Wren D, Walker BA, Brüggemann M, Catherwood MA, Pott C, Stamatopoulos K, et al. Comprehensive translocation and clonality detection in lymphoproliferative disorders by next-generation sequencing. Haematologica. 2017;102(2):e57–60.
92. Sørensen CD, Jørgensen JM, Nederby L, Hokland P, Nyvold CG. Common consensus LNA probe for quantitative PCR assays in cancer: vehicles for minimal residual disease detection in t(11;14) and t(14;18) positive malignant lymphomas. J Immunol Methods. 2014;406:131–6.
93. Hoster E, Pott C. Minimal residual disease in mantle cell lymphoma: insights into biology and impact on treatment. Hematology Am Soc Hematol Educ Program. 2016;2016(1):437–45.
94. Pott C, Hoster E, Delfau-Larue MH, Beldjord K, Böttcher S, Asnafi V, et al. Molecular remission is an independent predictor of clinical outcome in patients with mantle cell lymphoma after combined immunochemotherapy: a European MCL intergroup study. Blood. 2010;115(16):3215–23.
95. Böttcher S, Ritgen M, Buske S, Gesk S, Klapper W, Hoster E, et al. Minimal residual disease detection in mantle cell lymphoma: methods and significance of four-color flow cytometry compared to consensus IGH-polymerase chain reaction at initial staging and for follow-up examinations. Haematologica. 2008;93(4):551–9.
96. Cheminant M, Derrieux C, Touzart A, Schmit S, Grenier A, Trinquand A, et al. Minimal residual disease monitoring by 8-color flow cytometry in mantle cell lymphoma: An EU-MCL and LYSA study. Haematologica. 2016;101(3):336–45.
97. Avet-Loiseau H, Lauwers-Cances V, Corre J, Moreau P, Attal M, Munshi N. Minimal residual disease in multiple myeloma: final analysis of the IFM2009 trial. Blood. 2017;130:435.

98. Ho C, Arcila ME. Minimal residual disease detection of myeloma using sequencing of immunoglobulin heavy chain gene VDJ regions. Semin Hematol. 2018;55(1):13–8.
99. Landgren O. MRD testing in multiple myeloma: from a surrogate marker of clinical outcomes to an every-Day clinical tool. Semin Hematol. 2018;55(1):1–3.
100. Pott C, Brüggemann M, Ritgen M, van der Velden VHJ, van Dongen JJM, Kneba M. MRD detection in B-cell non-Hodgkin lymphomas using Ig gene rearrangements and chromosomal translocations as targets for real-time quantitative PCR. Methods Mol Biol. 1956;2019:199–228.
101. Camus V, Sarafan-Vasseur N, Bohers E, Dubois S, Mareschal S, Bertrand P, et al. Digital PCR for quantification of recurrent and potentially actionable somatic mutations in circulating free DNA from patients with diffuse large B-cell lymphoma. Leuk Lymphoma. 2016;57(9):2171–9.
102. Drandi D, Ferrero S, Ladetto M. Droplet digital PCR for minimal residual disease detection in mature lymphoproliferative disorders. Methods Mol Biol. 1768;2018:229–56.
103. Trotman J, Davies A, Hiddemann W, Hoster E, Marcus R, Schmidt C, et al. Relationship between MRD and PET responses and PFS in previously untreated follicular lymphoma in the GALLIUM trial. J Clin Oncol. 2018;36(15):7557.
104. Liu H, Johnson JL, Koval G, Malnassy G, Sher D, Damon LE, et al. Detection of minimal residual disease following induction immunochemotherapy predicts progression free survival in mantle cell lymphoma: final results of CALGB 59909. Haematologica. 2012;97(4):579–85.
105. Gressin R, Daguindau N, Tempescul A, Moreau A, Carras S, Tchernonog E, et al. A phase 2 study of rituximab, bendamustine, bortezomib and dexamethasone for first-line treatment of older patients with mantle cell lymphoma. Haematologica. 2019;104(1):138–46.
106. Chase ML, Armand P. Minimal residual disease in non-Hodgkin lymphoma – current applications and future directions. Br J Haematol. 2018;180(2):177–88.
107. Herrera AF, Armand P. Minimal residual disease assessment in lymphoma: methods and applications. J Clin Oncol. 2017;35(34):3877–87.
108. Jacob J, Kelsoe G, Rajewsky K, Weiss U. Intraclonal generation of antibody mutants in germinal centres. Nature. 1991;354:389–92.
109. Bahler DW, Levy R. Clonal evolution of a follicular lymphoma: evidence for antigen selection. Proc Natl Acad Sci U S A. 1992;89:6770–4.
110. Scherer F, Kurtz DM, Newman AM, Stehr H, Craig AF, Esfahani MS, et al. Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. Sci Transl Med. 2016;8(364):364ra155.
111. Okosun J, Bödör C, Wang J, Araf S, Yang CY, Pan C, et al. Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma. Nat Genet. 2014 Feb;46(2):176–81.
112. Korfi K, Ali S, Heward JA, Fitzgibbon J. Follicular lymphoma, a B cell malignancy addicted to epigenetic mutations. Epigenetics. 2017;12(5):370–7.
113. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. Nature. 2011;471(7337):189–95.
114. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature. 2011;476(7360):298–303.
115. Oricchio E, Nanjangud G, Wolfe AL, Schatz JH, Mavrakis KJ, Jiang M, et al. The Eph-receptor A7 is a soluble tumor suppressor for follicular lymphoma. Cell. 2011;147(3):554–64.
116. Pon JR, Wong J, Saberi S, Alder O, Moksa M, Grace Cheng SW, et al. MEF2B mutations in non-Hodgkin lymphoma dysregulate cell migration by decreasing MEF2B target gene activation. Nat Commun. 2015;6:7953.
117. Rossi D, Berra E, Cerri M, Deambrogi C, Barbieri C, Franceschetti S, et al. Aberrant somatic hypermutation in transformation of follicular lymphoma and chronic lymphocytic leukemia to diffuse large B-cell lymphoma. Haematologica. 2006;91(10):1405–9.

118. Xerri L, Bachy E, Fabiani B, Canioni D, Chassagne-Clément C, Dartigues-Cuilléres P, et al. Identification of MUM1 as a prognostic immunohistochemical marker in follicular lymphoma using computerized image analysis. Hum Pathol. 2014;45(10):2085–93.
119. Sweetenham JW, Goldman B, LeBlanc ML, Cook JR, Tubbs RR, Press OW, et al. Prognostic value of regulatory T cells, lymphoma-associated macrophages, and MUM-1 expression in follicular lymphoma treated before and after the introduction of monoclonal antibody therapy: a Southwest Oncology Group Study. Ann Oncol. 2010;21(6):1196–202.
120. Alhejaily A, Day AG, Feilotter HE, Baetz T, Lebrun DP. Inactivation of the CDKN2A tumor-suppressor gene by deletion or methylation is common at diagnosis in follicular lymphoma and associated with poor clinical outcome. Clin Cancer Res. 2014;20(6):1676–86.
121. Karube K, Martínez D, Royo C, Navarro A, Pinyol M, Cazorla M, et al. Recurrent mutations of NOTCH genes in follicular lymphoma identify a distinctive subset of tumours. J Pathol. 2014 Nov;234(3):423–30.
122. Berget E, Molven A, Løkeland T, Helgeland L, Vintermyr OK. IGHV gene usage and mutational status in follicular lymphoma: correlations with prognosis and patient age. Leuk Res. 2015;39(7):702–8.
123. García-Álvarez M, Alonso-Álvarez S, Prieto-Conde I, Jiménez C, Sarasquete ME, Chillón MC, et al. Immunoglobulin gene rearrangement IGHV3-48 is a predictive marker of histological transformation into aggressive lymphoma in follicular lymphomas. Blood Cancer J. 2019;9(7):52.
124. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger AM, Szczepanowski M, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. Lancet Oncol. 2015;16(9):1111–22.
125. Ahmed M, Zhang L, Nomie K, Lam L, Wang M. Gene mutations and actionable genetic lesions in mantle cell lymphoma. Oncotarget. 2016;7(36):58638–48.
126. Beà S, Valdés-Mas R, Navarro A, Salaverria I, Martín-Garcia D, Jares P, et al. Landscape of somatic mutations and clonal evolution in mantle cell lymphoma. Proc Natl Acad Sci U S A. 2013;110(45):18250–5.
127. Greiner TC, Dasgupta C, Ho VV, Weisenburger DD, Smith LM, Lynch JC, et al. Mutation and genomic deletion status of ataxia telangiectasia mutated (ATM) and p53 confer specific gene expression profiles in mantle cell lymphoma. Proc Natl Acad Sci U S A. 2006;103(7):2352–7.
128. Zhang J, Jima D, Moffitt AB, Liu Q, Czader M, Hsi ED, et al. The genomic landscape of mantle cell lymphoma is related to the epigenetically determined chromatin state of normal B cells. Blood. 2014;123(19):2988–96.
129. Rahal R, Frick M, Romero R, Korn JM, Kridel R, Chan FC, et al. Pharmacological and genomic profiling identifies NF-κB-targeted treatment strategies for mantle cell lymphoma. Nat Med. 2014;20(1):87–92.
130. Kridel R, Meissner B, Rogic S, Boyle M, Telenius A, Woolcock B, et al. Whole transcriptome sequencing reveals recurrent NOTCH1 mutations in mantle cell lymphoma. Blood. 2012;119(9):1963–71.
131. Meissner B, Kridel R, Lim RS, Rogic S, Tse K, Scott DW, et al. The E3 ubiquitin ligase UBR5 is recurrently mutated in mantle cell lymphoma. Blood. 2013;121(16):3161–4.
132. Rossi D, Ferrero S, Bruscaggin A, Ghione P, Di Rocco A, Spina V, et al. A molecular model for the prediction of progression free survival in Young mantle cell lymphoma patients treated with cytarabine-based high dose sequential chemotherapy and autologous stem cell transplantation: results from the MCL0208 phase III trial from Fondazione Italiana Linfomi (FIL). Blood. 2015;126:336.
133. Greiner TC, Moynihan MJ, Chan WC, Lytle DM, Pedersen A, Anderson JR, et al. p53 mutations in mantle cell lymphoma are associated with variant cytology and predict a poor prognosis. Blood. 1996;87(10):4302–10.
134. Camacho E, Hernández L, Hernández S, Tort F, Bellosillo B, Bosch F, et al. ATM gene inactivation in mantle cell lymphoma mainly occurs by truncating mutations and missense mutations involving the phosphatidylinositol-3 kinase domain and is associated with increasing numbers of chromosomal imbalances. Blood. 2002;99(1):238–44.

135. Weston VJ, Oldreive CE, Skowronska A, Oscier DG, Pratt G, Dyer MJS, et al. The PARP inhibitor olaparib induces significant killing of ATM-deficient lymphoid tumor cells *in vitro* and *in vivo*. Blood. 2010;116:4578–87.
136. Moding EJ, Lee CL, Castle KD, Oh P, Mao L, Zha S, et al. Atm deletion with dual recombinase technology preferentially radiosensitizes tumor endothelium. J Clin Invest. 2014;124(8):3325–38.
137. Haque W, Voong KR, Shihadeh F, Arzu I, Pinnix C, Mazloom A, et al. Radiation therapy is an effective modality in the treatment of mantle cell lymphoma, even in heavily pretreated patients. Clin Lymphoma Myeloma Leuk. 2014;14(6):474–9.
138. Hoster E, Rosenwald A, Berger F, Bernd HW, Hartmann S, Loddenkemper C, et al. Prognostic value of Ki-67 index, cytology, and growth pattern in mantle-cell lymphoma: results from randomized trials of the European mantle cell lymphoma network. J Clin Oncol. 2016;34(12):1386–94.
139. Geisler CH, Kolstad A, Laurell A, Räty R, Jerkeman M, Eriksson M, et al. The Mantle cell lymphoma international prognostic index (MIPI) is superior to the international prognostic index (IPI) in predicting survival following intensive first-line immunochemotherapy and autologous stem cell transplantation (ASCT). Blood. 2010;115(8):1530–3.
140. Delfau-Larue MH, Klapper W, Berger F, Jardin F, Briere J, Salles G, et al. High-dose cytarabine does not overcome the adverse prognostic value of CDKN2A and TP53 deletions in mantle cell lymphoma. Blood. 2015;126(5):604–11.
141. Eskelund CW, Dahl C, Hansen JW, Westman M, Kolstad A, Pedersen LB, et al. *TP53* mutations identify younger mantle cell lymphoma patients who do not benefit from intensive chemoimmunotherapy. Blood. 2017;130(17):1903–10.
142. Weng AP, Ferrando AA, Lee W, Morris JP 4th, Silverman LB, Sanchez-Irizarry C, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science. 2004;306(5694):269–71.
143. Thompson BJ, Buonamici S, Sulis ML, Palomero T, Vilimas T, Basso G, et al. The SCFFBW7 ubiquitin ligase complex as a tumor suppressor in T cell leukemia. J Exp Med. 2007;204(8):1825–35.
144. Yang P, Zhang W, Wang J, Liu Y, An R, Jing H. Genomic landscape and prognostic analysis of mantle cell lymphoma. Cancer Gene Ther. 2018;25(5–6):129–40.
145. Pham LV, Tamayo AT, Yoshimura LC, Lo P, Ford RJ. Inhibition of constitutive NF-kappa B activation in mantle cell lymphoma B cells leads to induction of cell cycle arrest and apoptosis. J Immunol. 2003;171(1):88–95.
146. Wu C, de Miranda NF, Chen L, Wasik AM, Mansouri L, Jurczak W, et al. Genetic heterogeneity in primary and relapsed mantle cell lymphomas: impact of recurrent CARD11 mutations. Oncotarget. 2016;7(25):38180–90.
147. Chen RW, Bemis LT, Amato CM, Myint H, Tran H, Birks DK, et al. Truncation in CCND1 mRNA alters miR-16-1 regulation in mantle cell lymphoma. Blood. 2008;112(3):822–9.
148. Mohanty A, Sandoval N, Das M, Pillai R, Chen L, Chen RW, et al. CCND1 mutations increase protein stability and promote ibrutinib resistance in mantle cell lymphoma. Oncotarget. 2016;7(45):73558–72.
149. Wiestner A, Tehrani M, Chiorazzi M, Wright G, Gibellini F, Nakayama K, et al. Point mutations and genomic deletions in CCND1 create stable truncated cyclin D1 mRNAs that are associated with increased proliferation rate and shorter survival. Blood. 2007;109(11):4599–606.
150. Orchard J, Garand R, Davis Z, Babbage G, Sahota S, Matutes E. A subset of t(11;14) lymphoma with mantle cell features displays mutated IgVH genes and includes patients with good prognosis, nonnodal disease. Blood. 2003;101:4975–81.
151. Welzel N, Le T, Marculescu R, Mitterbauer G, Chott A, Pott C, et al. Templated nucleotide addition and immunoglobulin JH-gene utilization in t(11;14) junctions: implications for the mechanism of translocation and the origin of mantle cell lymphoma. Cancer Res. 2001;61(4):1629–36.
152. Kienle D, Kröber A, Katzenberger T, Ott G, Leupolt E, Barth TF, et al. VH mutation status and VDJ rearrangement structure in mantle cell lymphoma: correlation with genomic aberrations, clinical characteristics, and outcome. Blood. 2003;102(8):3003–9.

153. Walsh SH, Thorsélius M, Johnson A, Söderberg O, Jerkeman M, Björck E, et al. Mutated VH genes and preferential VH3-21 use define new subsets of mantle cell lymphoma. Blood. 2003;101(10):4047–54.
154. Camacho FI, Algara P, Rodríguez A, Ruíz-Ballesteros E, Mollejo M, Martínez N, et al. Molecular heterogeneity in MCL defined by the use of specific VH genes and the frequency of somatic mutations. Blood. 2003;101(10):4042–6.
155. Fernàndez V, Salamero O, Espinet B, Solé F, Royo C, Navarro A, et al. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. Cancer Res. 2010;70(4):1408–18.
156. Scott DW, Abrisqueta P, Wright GW, Slack GW, Mottok A, Villa D, et al. New molecular assay for the proliferation signature in mantle cell lymphoma applicable to formalin-fixed paraffin-embedded biopsies. J Clin Oncol. 2017;35(15):1668–77.
157. Iqbal J, Shen Y, Liu Y, Fu K, Jaffe ES, Liu C, et al. Genome-wide miRNA profiling of mantle cell lymphoma reveals a distinct subgroup with poor prognosis. Blood. 2012;119(21):4939–48.
158. Navarro A, Clot G, Prieto M, Royo C, Vegliante MC, Amador V, et al. microRNA expression profiles identify subtypes of mantle cell lymphoma with different clinicobiological characteristics. Clin Cancer Res. 2013;19(12):3121–9.
159. Navarro A, Bea S, Fernandez V, Prieto M, Salaverria I, Jares P, et al. MicroRNA expression, chromosomal alterations, and immunoglobulin variable heavy chain hypermutations in Mantle cell lymphomas. Cancer Res. 2009;69(17):7071–8.
160. Husby S, Ralfkiaer U, Garde C, Zandi R, Ek S, Kolstad A, et al. miR-18b overexpression identifies mantle cell lymphoma patients with poor outcome and improves the MIPI-B prognosticator. Blood. 2015;125(17):2669–77.

Chapter 5
Follicular Lymphoma: Therapeutics and Management

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Introduction

Follicular lymphoma (FL) is the second most common form of non-Hodgkin lymphoma (NHL) and the most common lymphoma with an indolent clinical course. It is generally regarded as incurable; however in the modern era, the prognosis is often favorable with many patients achieving long-term disease control. Management is largely dependent on disease stage, presence or absence of symptoms, and overall disease burden. As such, patients can be categorized into groups with limited-stage disease (stages I or II) or advanced stage disease (stages III or IV). Treatment approaches vary significantly between these groups and can range from surveillance alone to radiation therapy or chemoimmunotherapy and radioimmunotherapy. The treatment strategies presented here pertain to patients with histologic grade 1, 2, or 3a FL; patients with grade 3b FL are best treated according to guidelines for more clinically aggressive B-cell lymphomas (e.g., diffuse large B-cell lymphoma), as their course and prognosis more closely mirror them than low-grade FL [1].

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Management of Limited-Stage Follicular Lymphoma

Limited-stage FL is relatively uncommon, as most cases of FL are diagnosed at more advanced stages, and there are therefore no randomized studies to support an optimal management strategy. Instead, we rely on observational data and expert opinion to guide our treatment approach. External beam radiation is often considered in this patient population as several studies have demonstrated long-term disease-free survival and potential cure with this approach, with up to 40–50% of patients free of disease at 10 years [2–4]. Given the excellent outcomes in this population, efforts have been made to obviate the risk of radiation-related complications, and involved-site radiation (ISRT) has become the standard of care.

If significant morbidity from ISRT is a concern, then a period of surveillance may be a reasonable alternative approach, particularly in those with a limited life expectancy. This was demonstrated in a small trial that showed patients with limited-stage disease who received no initial therapy had an estimated 10-year survival of 85% with more than 50% of patients remaining untreated at 6 years [5]. A recent study utilizing the LymphoCare database shed light on more contemporary practice patterns and associated outcomes. Rituximab-containing regimens were used most commonly with 40% of patients receiving either rituximab with chemotherapy or rituximab alone. The remainder of patients received the following: 27% radiation, 17% observation, 13% combined modality therapy, and 3% other. Treatment strategies utilizing systemic therapy led to improved progression-free survival (PFS), but no difference in overall survival (OS) was seen with any of the approaches [6]. It is also important to highlight that proper staging with a computed tomography (CT) scan, and bone marrow biopsy is required to demonstrate I-II disease prior to localized treatment.

These findings suggest that several treatment options may be appropriate, and optimal management should be personalized according to patient-specific factors (e.g., bulky disease, age, comorbidities, and goals of care). Current guidelines from the National Comprehensive Cancer Network (NCCN) recommend upfront ISRT for non-bulky (less than 7 cm) stage I or localized stage II disease; observation alone is an option if there are concerns about radiation toxicity. For bulky disease (7 cm or larger), anti-CD20 monoclonal antibody monotherapy or combination chemoimmunotherapy is preferred; however, combined modality therapy or observation alone can be considered.

Management of Advanced Stage Follicular Lymphoma

The majority of patients with FL present with advanced stage disease which cannot be cured with conventional treatments. The disease is often responsive to therapy, however, and there are a multitude of effective treatment options. As such, many patients receive multiple treatments over the span of years, often separated by long

periods of remission. In determining management for newly diagnosed patients with FL, we favor an individualized approach which takes into consideration the presence or absence of symptoms, extent of tumor burden, patient's age, medical comorbidities, as well as goals of care. The Groupe d'Etude des Lymphomes Folliculaires (GELF) criteria (Table 5.1) is the most widely used approach for assessing tumor burden and can be helpful in guiding management decisions about whether patients require treatment. Patients who meet any of the GELF criteria are considered to have high tumor burden.

In conjunction with the GELF criteria, patients can be stratified into four categories: (1) asymptomatic with low tumor burden, (2) asymptomatic with high tumor burden, (3) symptomatic with low tumor burden, and (4) symptomatic with high tumor burden. A broad summary of treatment approaches for these groups is shown in Table 5.2.

Asymptomatic Disease with Low Tumor Burden

Unlike early-stage and localized follicular lymphoma, advanced stage disease often requires upfront systemic treatment, though the decision of whether to treat continues to be based on individual patient factors including presence of symptoms, tumor burden, patient age and comorbidities, and the patient's goals for therapy. Advanced stage disease may be low-grade (grade 1–2) or higher grade (grade 3a) and is not curable but is manageable due to the highly chemotherapy-responsive nature of the disease.

Table 5.1 GELF criteria [7]

Involvement of ≥3 nodal sites, each with a diameter of ≥3 cm
Any nodal or extranodal tumor mass with a diameter of ≥7 cm
B symptoms
Splenomegaly
Pleural effusions or peritoneal ascites
Cytopenias (leukocytes $<1.0 \times 10^9$/L and/or platelets $<100 \times 10^9$/L)
Leukemia ($>5.0 \times 10^9$/L malignant cells)

Table 5.2 Management approach in follicular lymphoma

	Low tumor burden	High tumor burden
Asymptomatic	Observation vs. Rituximab monotherapy	Observation vs. Chemoimmunotherapy
Symptomatic	Rituximab monotherapy vs. Chemoimmunotherapy	Chemoimmunotherapy With or without Rituximab maintenance

Adapted from Kahl et al. [8]

There is a subset of patients even with advanced stage disease who may be appropriate candidates for a "watch-and-wait" observation approach if they have a low burden of disease by GELF criteria and no constitutional symptoms. With this approach, patients are monitored regularly for symptoms related to FL, signs of organ compromise, and cytopenias. The observation strategy was supported by early retrospective analyses from Stanford University in the 1970s and 1980s which showed no detrimental effect on patient outcomes in appropriately selected patients [9, 10]. Several randomized trials have since confirmed this, the largest of which compared upfront therapy with chlorambucil to observation alone and found no difference in OS at 16 years [11]. Additional studies have shown that early treatment [7, 12] does not improve outcomes compared to observation.

One randomized trial did compare observation to single-agent rituximab with or without rituximab maintenance in patients with previously untreated asymptomatic FL with low tumor burden [13]. A statistically significant difference in PFS and time to first chemotherapy was observed in the rituximab groups; however, there was no difference in OS or incidence of histologic transformation. This study also looked at quality of life metrics as many patients, though asymptomatic, struggle with the diagnosis and uncertainty of this disease. They found that the prevalence of anxiety and depression is higher in this population compared to the general public and observed that patients labeled as "anxious" more readily adapted to their diagnosis when they underwent treatment rather than observation. Given the quality of life benefit, upfront rituximab monotherapy may be indicated for a subset of this population.

Overall, these data support the use of observation as the standard approach for patients with asymptomatic FL with low tumor burden. To date, no studies have demonstrated a survival benefit for upfront therapy; however, quality of life factors and other potential benefits of treatment (e.g., improved PFS and longer time to first chemotherapy) should be considered, and rituximab monotherapy may be appropriate in a subset of patients who particularly value these outcomes. In this group, retreatment with rituximab at the time of progression should be favored over rituximab maintenance.

Symptomatic Disease or High Tumor Burden

In patients with advanced disease who have constitutional symptoms at the time of diagnosis or who have high tumor burden, upfront therapy is most appropriate. Conventional cytotoxic chemotherapy has long been the standard of care in this setting, with the anti-CD20 monoclonal antibody rituximab added following its development in the 1990s. This represented a major advance in therapy for FL, as the addition of rituximab to chemotherapy allowed for improved response rates, PFS, and OS [14–16]. In addition, rituximab monotherapy can be used for patients with advanced stage but low-grade FL with overall response rates of about 70% and CR rates of about 30% [17–19]. The efficacy of single-agent rituximab was further

demonstrated in long-term follow-up from the SAKK trial, in which FL patients received four weekly doses of rituximab followed by randomization to observation versus four doses of maintenance rituximab given every 2 months [20]. Patients receiving rituximab maintenance had nearly doubled PFS of 23 versus 12 months, and nearly 45% remain in remission after 8 years of follow-up.

Chemotherapy backbones used with rituximab vary and most commonly include cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP); cyclophosphamide, vincristine, and prednisone (CVP); fludarabine, cyclophosphamide, and mitoxantrone (FCM); fludarabine and mitoxantrone (FM); and bendamustine (B).

The FOLL05 study conducted by the Fondazione Italiana Linfomi and published in 2013 [21] with long-term follow-up reported in 2018 [22] directly compared three of these regimens. R-CHOP and R-FM were shown to have improved outcomes (time to treatment failure and PFS, with no difference in OS) compared to R-CVP. R-CHOP was shown to have a better safety profile than R-FM, which had higher rates of grade 3 or 4 neutropenia than the other regimens. Based on this data, R-CHOP was adopted as the optimal regimen for first-line treatment of advanced stage FL.

More recently bendamustine, an alkylating agent, has also been adopted for use in this setting together with rituximab (BR). A phase III conducted by the Study group indolent Lymphoma (StiL) [23] compared BR to R-CHOP in patients with indolent non-Hodgkin and mantle cell lymphoma with high tumor burden and found similar overall response rates between the two groups (91–92%) but a significantly higher complete response (CR) rate of 39.8% in the BR group compared to 30.0% in the R-CHOP group. A sub-analysis of FL patients showed longer median PFS after treatment with BR compared to R-CHOP as well, with no difference in OS. The BR regimen was also associated with fewer adverse events including hematologic toxicity, alopecia, infection, peripheral neuropathy, and stomatitis, though drug-associated skin reactions were more common. These findings were confirmed in the BRIGHT trial [24], a phase III study that found BR to be non-inferior to standard therapy (R-CHOP or R-CVP) in treatment-naïve patients with indolent non-Hodgkin or mantle cell lymphoma with primary endpoint of CR, and overall response rates were significantly higher with BR. This study again showed higher rates of hypersensitivity reactions in the BR group and higher incidence of peripheral neuropathy and alopecia in the R-CHOP and R-CVP groups. These results support the use of BR as an alternative to R-CHOP or R-CVP in patients with FL.

Another potential approach for first-line treatment of FL is rituximab combined with lenalidomide, an oral immunomodulatory agent given at a dose of 20 mg per day on days 1 to 21 of each 28-day cycle, up to 12 cycles in patients with clinical response. Initial results for this regimen were reported in 2014; a phase II study of 46 patients had an 87% CR rate and 11% partial response (PR) rate for an overall response rate of 98% [25]. Three-year PFS was 78.5%. Primary toxicities were neutropenia, muscle pain, and rash. The subsequent international phase III RELEVANCE trial compared the rituximab plus lenalidomide regimen to rituximab-based chemoimmunotherapy for initial management of FL; both groups received maintenance rituximab [26]. Outcomes including CR and 3-year PFS were similar

in both groups. Patients receiving rituximab-chemotherapy had higher rates of grade 3 or 4 neutropenia and neutropenic fever; patients receiving rituximab-lenalidomide had higher rates of grade 3 or 4 cutaneous reactions.

More recently, the international randomized phase III GALLIUM study compared obinutuzumab, a type II anti-CD20 monoclonal antibody, to rituximab when combined with chemotherapy (CHOP, bendamustine, or CVP) [27, 28]. Both 3-year PFS and time to next treatment were superior in the obinutuzumab arms compared to the rituximab arms (PFS 80% vs. 73%). There was increased toxicity in the obinutuzumab arms with the most common grade 3 to 5 adverse events being cytopenias (particularly neutropenia), infusion-related reactions, and pneumonia. Toxicities were also higher in the bendamustine arms. Due to opportunistic infections in the obinutuzumab plus bendamustine group, prophylactic antibiotics and infectious disease monitoring are highly recommended.

Response to treatment is typically assessed using [^{18}F] fluorodeoxyglucose (FDG) positron emission tomography (PET) and classified according to the Lugano criteria [29]. Studies have shown that responses by FDG-PET after four cycles of R-CHOP and at the end of therapy are predictive of 2-year PFS and OS, with significantly better outcomes seen in patients who are PET negative [30, 31]. A recent study also reported that assessment of minimal residual disease (MRD) status based on detection of the t(14;18) *BCL2/IGH* translocation in the bone marrow may correlate with long-term outcomes [32].

In summary, chemoimmunotherapy regimens comprised of anti-CD20 monoclonal antibodies in combination with a cytotoxic chemotherapy backbone are standard of care for first-line treatment of advanced stage FL in patients with symptomatic disease or high tumor burden. CHOP and bendamustine are the most commonly used chemotherapy regimens. The development of rituximab has changed the landscape of FL therapy; newer data suggests a possible benefit of obinutuzumab over rituximab, though with concern for increased toxicity, and longer-term data will help to elucidate which patients are appropriate candidates for this.

Maintenance Therapy

The phase III PRIMA trial [33] sought to investigate whether maintenance rituximab after completion of first-line rituximab-based chemoimmunotherapy provides additional benefit. Patients underwent induction with R-CHOP (75%), R-CVP (22%), or R-FCM (3%) and then were randomized to either observation or single-dose rituximab every 2 months for 2 years. After 3 years of follow-up, there was a significantly higher 2-year PFS in the maintenance arm (75%) compared to the observation arm (58%), with the beneficial effect seen regardless of which induction regimen was used. There was no difference in OS. However, there was a higher incidence of grade 3 or 4 adverse events in the group receiving maintenance therapy. These mixed findings suggest a role for rituximab maintenance in some patients, though used with caution given the increase in toxicity and lack of survival benefit.

Histologic Transformation

Patients with FL are at risk for transformation to more aggressive B-cell lymphomas, most commonly diffuse large B-cell lymphoma (DLBCL). Putative mechanisms for transformation include direct clonal evolution of an aggressive subclone of FL cells or divergent evolution from a more immature common progenitor cell [34]. Additional acquired genetic abnormalities have been implicated in the transformation process, including upregulation of *MYC* expression [35, 36] and mutations in the *TP53* gene [37]. As a result, the many FL cases that transform to DLBCL are double-hit lymphomas harboring translocations affecting *MYC* as well as either the *BCL2* or *BCL6* genes [38].

The rate of transformation is approximately 3% per year [39] and persists through the disease course. Factors associated with higher incidence of histologic transformation include advanced stage and high-risk Follicular Lymphoma International Prognostic Index (FLIPI) or International Prognostic Index (IPI) score at diagnosis [40]. Histologic transformation is associated with shorter PFS and OS with median survival from transformation of 1–2 years historically [39, 40], though more recent reports in the post-rituximab era suggest survival of up to 5 years after transformation [41]. Transformation risk is no different between FL patients treated with R-CHOP or R-CVP regimens, though use of rituximab maintenance therapy has been shown to decrease transformation risk [41].

Once transformed, treatment should follow guidelines for the more aggressive histologic subtype and often consists of intensive chemoimmunotherapy regimens. An evaluation of patients in the PRIMA study who transformed from FL to aggressive B-cell lymphoma showed lower rates of CR and higher rates of disease progression with salvage therapy than non-transformed patients, and outcomes were improved with autologous hematopoietic stem cell transplantation (HSCT) [42].

Treatment of Relapsed or Refractory Disease

Following induction chemoimmunotherapy, with or without maintenance therapy, patients with FL may experience refractory disease. Others relapse following initial treatment response and a variable interval with minimal disease manifestations. When this occurs, there are multiple treatment approaches to consider. Important factors impacting when and how to treat relapsed or refractory disease include prior treatments a patient received, duration of prior response, age, comorbid medical illnesses, and the patient's goals for therapy [8].

For patients with rituximab-refractory FL, bendamustine is an FDA-approved option with a regimen of single-agent bendamustine 120 mg/m^2 IV on days 1 and 2 of a 21-day cycle. This was based on a phase III multicenter study reporting an overall response rate of 75% and median PFS of 9.3 months [43]. Due to cumulative myelosuppression commonly seen with this regimen, about two-thirds of patients in

this trial required dose modifications or delays. Bendamustine can also be used in combination with rituximab, though at lower doses of 70–90 mg/m^2 on days 1 and 2 of a 28-day cycle.

Additional approaches for treatment of relapsed or refractory follicular lymphoma include:

- Single-agent rituximab, though rituximab-refractory disease becomes an increasing concern once patients have received multiple lines of therapy or rituximab maintenance. However, this is well-tolerated and may be useful in patients who cannot tolerate cytotoxic chemotherapy.
- Fludarabine-based regimens in patients who have relapsed after prior treatment with an alkylator-based regimen. Due to risk of immunosuppression, these therapies should be used with caution in elderly or heavily pretreated patients [8].
- Bendamustine in combination with obinutuzumab, which was shown in the GADOLIN trial [44, 45] to significantly improve PFS and OS compared to bendamustine alone.
- Radioimmunotherapy in patients with non-bulky disease and minimal or no bone marrow involvement. Treatment with [90]yttrium-ibritumomab-tiuxetan has shown response rates up to 80% with 14-month duration of response [46, 47]. It can also be used for consolidation following induction chemotherapy where it showed improvement in PFS compared to observation [48]. The primary toxicity is reversible myelosuppression. A second radioimmunotherapy agent [131]I-tositumomab is no longer available [38].
- Idelalisib, a first-in-class inhibitor of phosphatidylinositol 3-kinase (PI3K) delta, targets an enzyme downstream of the B-cell receptor. A phase II study in patients with indolent NHL refractory to both rituximab and an alkylating agent showed a response rate of 57% for patients treated with idelalisib 150 mg twice daily, with median duration of response of 12.5 months [49]. The highest-grade toxicities were neutropenia (27%), transaminase elevations (13%), diarrhea (13%), and pneumonia (7%). It received accelerated FDA approval in 2014. Development in the first line was halted, however, due to increased risk of death including *Pneumocystis carinii* and cytomegalovirus infections [38]. An additional PI3K inhibitor, copanlisib, has been FDA-approved for FL, with other next-generation PI3K inhibitors under FDA review [50].

Additionally, numerous novel agents are currently under development or investigation for use in treatment of relapsed or refractory follicular lymphoma. These include [8, 38]:

- PI3K inhibitors (including umbralisib, duvelisib)
- Immunomodulatory agents (including lenalidomide)
- Proteasome inhibitors (including bortezomib)
- BTK inhibitors (including ibrutinib)
- BCL2 inhibitors (including venetoclax)
- Antibody-drug conjugates (including polatuzumab vedotin, inotuzumab ozogamicin)

- Novel anti-CD20 monoclonal antibodies (including obinutuzumab)
- Immune checkpoint inhibitors (including nivolumab, pidilizumab)
- Inhibitors of nuclear export proteins
- Chimeric antigen receptor T-cell therapy (CAR T)

Role of Hematopoietic Stem Cell Transplantation

Both high-dose chemotherapy with autologous HSCT and allogeneic HSCT have been studied for treatment of follicular lymphoma. These therapies are most appropriate for patients who are of younger age and with higher-risk disease features, including brief remission with prior therapy before disease relapse.

A review of data from the International Bone Marrow Transplant Registry including over 900 patients who underwent autologous HSCT (81%) or allogeneic HSCT (19%) for FL demonstrated that durable remissions can be achieved with either approach [51]. Allogeneic HSCT had the highest treatment-related mortality rates (30%) but also the lowest 5-year recurrence rate (21%). Overall 5-year survival rates were 51% with allogeneic HSCT and 62% with purged autologous HSCT. Factors associated with adverse outcomes included advanced age, prolonged interval from diagnosis to transplantation, high lactate dehydrogenase (LDH), refractory disease, bone marrow involvement, and poor performance status. Most recently, non-myeloablative reduced-intensity conditioning (RIC) regimens for allogeneic HSCT have been utilized in an effort to reduce treatment-related mortality, and studies have shown higher survival rates with this approach [52, 53].

Autologous HSCT was compared to chemotherapy for treatment of relapsed FL in a small randomized trial conducted in the pre-rituximab era [54]. The study found improved PFS with autologous HSCT with a trend toward improved OS but no statistically significant difference. A long-term follow-up report of 121 patients treated with myeloablative chemotherapy followed by autologous HSCT showed that nearly half of patients were still in remission after 10–15 years, suggesting that some of these patients may be effectively cured [55]. The report also showed that outcomes were significantly better for patients treated in second remission compared to later in the disease course. Data do not support the use of autologous HSCT in first remission for FL patients [56, 57]. A 2012 meta-analysis of autologous HSCT used in initial management of FL showed improvement in PFS but not OS when compared to conventional chemotherapy [58].

Conclusions

Follicular lymphoma is a common form of indolent NHL, and the approach to management is largely based on presence of symptoms, extent of tumor burden, and other patient-specific factors. Patients with localized early-stage disease can often

be cured with involved-site radiation. In a subset of patients, treatment with rituximab monotherapy may be appropriate. In patients with more advanced disease, upfront therapy is often indicated. Various approaches including anti-CD20 monoclonal antibody monotherapy or combination chemoimmunotherapy can be utilized. Maintenance therapy may be beneficial as well. Relapsed or refractory FL can be treated with similar chemoimmunotherapy regimens or with a number of more novel agents and approaches. HSCT is also an option in appropriately selected patients with high-risk disease.

References

1. Wahlin BE, Yri OE, Kimby E, et al. Clinical significance of the WHO grades of follicular lymphoma in a population-based cohort of 505 patients with long follow-up times. Br J Haematol. 2012;156(2):225–33. https://doi.org/10.1111/j.1365-2141.2011.08942.x.
2. Guadagnolo BA, Li S, Neuberg D, et al. Long-term outcome and mortality trends in early-stage, Grade 1-2 follicular lymphoma treated with radiation therapy. Int J Radiat Oncol Biol Phys. 2006;64(3):928–34. https://doi.org/10.1016/j.ijrobp.2005.08.010.
3. Wilder RB, Jones D, Tucker SL, et al. Long-term results with radiotherapy for Stage I-II follicular lymphomas. Int J Radiat Oncol Biol Phys. 2001;51(5):1219–27. https://doi.org/10.1016/s0360-3016(01)01747-3.
4. Mac Manus MP, Hoppe RT. Is radiotherapy curative for stage I and II low-grade follicular lymphoma? Results of a long-term follow-up study of patients treated at Stanford University. J Clin Oncol Off J Am Soc Clin Oncol. 1996;14(4):1282–90. https://doi.org/10.1200/JCO.1996.14.4.1282.
5. Advani R, Rosenberg SA, Horning SJ. Stage I and II follicular non-Hodgkin's lymphoma: long-term follow-up of no initial therapy. J Clin Oncol Off J Am Soc Clin Oncol. 2004;22(8):1454–9. https://doi.org/10.1200/JCO.2004.10.086.
6. Friedberg JW, Byrtek M, Link BK, et al. Effectiveness of first-line management strategies for stage I follicular lymphoma: analysis of the National LymphoCare Study. J Clin Oncol Off J Am Soc Clin Oncol. 2012;30(27):3368–75. https://doi.org/10.1200/JCO.2011.40.6546.
7. Brice P, Bastion Y, Lepage E, et al. Comparison in low-tumor-burden follicular lymphomas between an initial no-treatment policy, prednimustine, or interferon alfa: a randomized study from the Groupe d'Etude des Lymphomes Folliculaires. Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol Off J Am Soc Clin Oncol. 1997;15(3):1110–7. https://doi.org/10.1200/JCO.1997.15.3.1110.
8. Kahl BS, Yang DT. Follicular lymphoma: evolving therapeutic strategies. Blood. 2016;127(17):2055–63. https://doi.org/10.1182/blood-2015-11-624288.
9. Portlock CS. No initial therapy for stage III and IV non-Hodgkin's lymphomas of favorable histologic types. Ann Intern Med. 1979;90(1):10. https://doi.org/10.7326/0003-4819-90-1-10.
10. Horning SJ, Rosenberg SA. The natural history of initially untreated low-grade non-Hodgkin's lymphomas. N Engl J Med. 1984;311(23):1471–5. https://doi.org/10.1056/NEJM198412063112303.
11. Ardeshna KM, Smith P, Norton A, et al. Long-term effect of a watch and wait policy versus immediate systemic treatment for asymptomatic advanced-stage non-Hodgkin lymphoma: a randomised controlled trial. Lancet. 2003;362(9383):516–22. https://doi.org/10.1016/s0140-6736(03)14110-4.
12. Young RC, Longo DL, Glatstein E, Ihde DC, Jaffe ES, DeVita VT. The treatment of indolent lymphomas: watchful waiting v aggressive combined modality treatment. Semin Hematol. 1988;25(2 Suppl 2):11–6.

13. Ardeshna KM, Qian W, Smith P, et al. Rituximab versus a watch-and-wait approach in patients with advanced-stage, asymptomatic, non-bulky follicular lymphoma: an open-label randomised phase 3 trial. Lancet Oncol. 2014;15(4):424–35. https://doi.org/10.1016/S1470-2045(14)70027-0.
14. Hiddemann W. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood. 2005;106(12):3725–32. https://doi.org/10.1182/blood-2005-01-0016.
15. Marcus R, Imrie K, Solal-Celigny P, et al. Phase III study of R-CVP compared with cyclophosphamide, vincristine, and prednisone alone in patients with previously untreated advanced follicular lymphoma. J Clin Oncol. 2008;26(28):4579–86. https://doi.org/10.1200/JCO.2007.13.5376.
16. Herold M, Dölken G, Fiedler F, et al. Randomized phase III study for the treatment of advanced indolent non-Hodgkin's lymphomas (NHL) and mantle cell lymphoma: chemotherapy versus chemotherapy plus rituximab. Ann Hematol. 2003;82(2):77–9. https://doi.org/10.1007/s00277-002-0581-1.
17. Colombat P, Salles G, Brousse N, et al. Rituximab (anti-CD20 monoclonal antibody) as single first-line therapy for patients with follicular lymphoma with a low tumor burden: clinical and molecular evaluation. Blood. 2001;97(1):101–6. https://doi.org/10.1182/blood.v97.1.101.
18. Ghielmini M, Schmitz S-FH, Cogliatti SB, et al. Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly × 4 schedule. Blood. 2004;103(12):4416–23. https://doi.org/10.1182/blood-2003-10-3411.
19. Witzig TE, Vukov AM, Habermann TM, et al. Rituximab therapy for patients with newly diagnosed, advanced-stage, follicular grade I non-Hodgkin's lymphoma: a phase II trial in the North Central Cancer Treatment Group. J Clin Oncol Off J Am Soc Clin Oncol. 2005;23(6):1103–8. https://doi.org/10.1200/JCO.2005.12.052.
20. Martinelli G, Schmitz S-FH, Utiger U, et al. Long-term follow-up of patients with follicular lymphoma receiving single-agent rituximab at two different schedules in trial SAKK 35/98. J Clin Oncol Off J Am Soc Clin Oncol. 2010;28(29):4480–4. https://doi.org/10.1200/JCO.2010.28.4786.
21. Federico M, Luminari S, Dondi A, et al. R-CVP versus R-CHOP versus R-FM for the initial treatment of patients with advanced-stage follicular lymphoma: results of the FOLL05 trial conducted by the Fondazione Italiana Linfomi. J Clin Oncol. 2013;31(12):1506–13. https://doi.org/10.1200/JCO.2012.45.0866.
22. Luminari S, Ferrari A, Manni M, et al. Long-term results of the FOLL05 trial comparing R-CVP versus R-CHOP versus R-FM for the initial treatment of patients with advanced-stage symptomatic follicular lymphoma. J Clin Oncol. 2017;36(7):689–96. https://doi.org/10.1200/JCO.2017.74.1652.
23. Rummel MJ, Niederle N, Maschmeyer G, et al. Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. Lancet. 2013;381(9873):1203–10. https://doi.org/10.1016/S0140-6736(12)61763-2.
24. Flinn IW, van der Jagt R, Kahl BS, et al. Randomized trial of bendamustine-rituximab or R-CHOP/R-CVP in first-line treatment of indolent NHL or MCL: the BRIGHT study. Blood. 2014;123(19):2944–52. https://doi.org/10.1182/blood-2013-11-531327.
25. Fowler NH, Davis RE, Rawal S, et al. Safety, activity, and immune effects of lenalidomide and rituximab in untreated indolent lymphoma. Lancet Oncol. 2014;15(12):1311–8. https://doi.org/10.1016/S1470-2045(14)70455-3.
26. Morschhauser F, Fowler NH, Feugier P, et al. Rituximab plus Lenalidomide in advanced untreated follicular lymphoma. N Engl J Med. 2018;379(10):934–47. https://doi.org/10.1056/NEJMoa1805104.
27. Marcus R, Davies A, Ando K, et al. Obinutuzumab for the first-line treatment of follicular lymphoma. N Engl J Med. 2017;377(14):1331–44. https://doi.org/10.1056/NEJMoa1614598.

28. Hiddemann W, Barbui AM, Canales MA, et al. Immunochemotherapy with obinutuzumab or rituximab for previously untreated follicular lymphoma in the GALLIUM study: influence of chemotherapy on efficacy and safety. J Clin Oncol. 2018;36(23):2395–404. https://doi.org/10.1200/JCO.2017.76.8960.
29. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol Off J Am Soc Clin Oncol. 2014;32(27):3059–68. https://doi.org/10.1200/JCO.2013.54.8800.
30. Dupuis J, Berriolo-Riedinger A, Julian A, et al. Impact of [(18)F]fluorodeoxyglucose positron emission tomography response evaluation in patients with high-tumor burden follicular lymphoma treated with immunochemotherapy: a prospective study from the Groupe d'Etudes des Lymphomes de l'Adulte and GOELAMS. J Clin Oncol Off J Am Soc Clin Oncol. 2012;30(35):4317–22. https://doi.org/10.1200/JCO.2012.43.0934.
31. Luminari S, Biasoli I, Versari A, et al. The prognostic role of post-induction FDG-PET in patients with follicular lymphoma: a subset analysis from the FOLL05 trial of the Fondazione Italiana Linfomi (FIL). Ann Oncol Off J Eur Soc Med Oncol. 2014;25(2):442–7. https://doi.org/10.1093/annonc/mdt562.
32. Galimberti S, Luminari S, Ciabatti E, et al. Minimal residual disease after conventional treatment significantly impacts on progression-free survival of patients with follicular lymphoma: the FIL FOLL05 trial. Clin Cancer Res Off J Am Assoc Cancer Res. 2014;20(24):6398–405. https://doi.org/10.1158/1078-0432.CCR-14-0407.
33. Salles G, Seymour JF, Offner F, et al. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. Lancet. 2011;377(9759):42–51. https://doi.org/10.1016/S0140-6736(10)62175-7.
34. Carlotti E, Wrench D, Matthews J, et al. Transformation of follicular lymphoma to diffuse large B-cell lymphoma may occur by divergent evolution from a common progenitor cell or by direct evolution from the follicular lymphoma clone. Blood. 2009;113(15):3553–7. https://doi.org/10.1182/blood-2008-08-174839.
35. Lossos IS, Alizadeh AA, Diehn M, et al. Transformation of follicular lymphoma to diffuse large-cell lymphoma: alternative patterns with increased or decreased expression of c-myc and its regulated genes. Proc Natl Acad Sci U S A. 2002;99(13):8886–91. https://doi.org/10.1073/pnas.132253599.
36. Martinez-Climent JA, Alizadeh AA, Segraves R, et al. Transformation of follicular lymphoma to diffuse large cell lymphoma is associated with a heterogeneous set of DNA copy number and gene expression alterations. Blood. 2003;101(8):3109–17. https://doi.org/10.1182/blood-2002-07-2119.
37. Davies AJ, Lee AM, Taylor C, et al. A limited role for TP53 mutation in the transformation of follicular lymphoma to diffuse large B-cell lymphoma. Leukemia. 2005;19(8):1459–65. https://doi.org/10.1038/sj.leu.2403802.
38. Feugier P, Filliatre-Clement L. Recent advances in the first-line treatment of follicular non-Hodgkin lymphoma. F1000Research. 2019;8:294. https://doi.org/10.12688/f1000research.16686.1.
39. Al-Tourah AJ, Gill KK, Chhanabhai M, et al. Population-based analysis of incidence and outcome of transformed non-Hodgkin's lymphoma. J Clin Oncol. 2008;26(32):5165–9.
40. Montoto S, Davies AJ, Matthews J, et al. Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. J Clin Oncol. 2007;25(17):2426–33.
41. Wagner-Johnston ND, Link BK, Byrtek M, et al. Outcomes of transformed follicular lymphoma in the modern era: a report from the National LymphoCare Study (NLCS). Blood. 2015;126(7):851–7. https://doi.org/10.1182/blood-2015-01-621375.
42. Sarkozy C, Trneny M, Xerri L, et al. Risk factors and outcomes for patients with follicular lymphoma who had histologic transformation after response to first-line immunochemother-

apy in the PRIMA trial. J Clin Oncol Off J Am Soc Clin Oncol. 2016;34(22):2575–82. https://doi.org/10.1200/JCO.2015.65.7163.
43. Kahl BS, Bartlett NL, Leonard JP, et al. Bendamustine is effective therapy in patients with rituximab-refractory, indolent B-cell non-Hodgkin lymphoma. Cancer. 2010;116(1):106–14. https://doi.org/10.1002/cncr.24714.
44. Sehn LH, Chua N, Mayer J, et al. Obinutuzumab plus bendamustine versus bendamustine monotherapy in patients with rituximab-refractory indolent non-Hodgkin lymphoma (GADOLIN): a randomised, controlled, open-label, multicentre, phase 3 trial. Lancet Oncol. 2016;17(8):1081–93. https://doi.org/10.1016/S1470-2045(16)30097-3.
45. Cheson BD, Chua N, Mayer J, et al. Overall survival benefit in patients with rituximab-refractory indolent non-Hodgkin lymphoma who received obinutuzumab plus bendamustine induction and obinutuzumab maintenance in the GADOLIN study. J Clin Oncol. 2018;36(22):2259–66. https://doi.org/10.1200/JCO.2017.76.3656.
46. Witzig TE, Gordon LI, Cabanillas F, et al. Randomized controlled trial of Yttrium-90–Labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. J Clin Oncol. 2002;20(10):2453–63. https://doi.org/10.1200/JCO.2002.11.076.
47. Mondello P, Cuzzocrea S, Navarra M, Mian M. 90 Y-ibritumomab tiuxetan: a nearly forgotten opportunity. Oncotarget. 2015;7(7):7597–609. https://doi.org/10.18632/oncotarget.6531.
48. Morschhauser F, Radford J, Van Hoof A, et al. 90Yttrium-ibritumomab tiuxetan consolidation of first remission in advanced-stage follicular non-Hodgkin lymphoma: updated results after a median follow-up of 7.3 years from the International, Randomized, Phase III First-LineIndolent trial. J Clin Oncol Off J Am Soc Clin Oncol. 2013;31(16):1977–83. https://doi.org/10.1200/JCO.2012.45.6400.
49. Gopal AK, Kahl BS, de Vos S, et al. PI3Kδ inhibition by idelalisib in patients with relapsed indolent lymphoma. N Engl J Med. 2014;370(11):1008–18. https://doi.org/10.1056/NEJMoa1314583.
50. Dreyling M, Morschhauser F, Bouabdallah K, et al. Phase II study of copanlisib, a PI3K inhibitor, in relapsed or refractory, indolent or aggressive lymphoma. Ann Oncol Off J Eur Soc Med Oncol. 2017;28(9):2169–78. https://doi.org/10.1093/annonc/mdx289.
51. van Besien K, Loberiza FR, Bajorunaite R, et al. Comparison of autologous and allogeneic hematopoietic stem cell transplantation for follicular lymphoma. Blood. 2003;102(10):3521–9. https://doi.org/10.1182/blood-2003-04-1205.
52. Rezvani AR, Storer B, Maris M, et al. Nonmyeloablative allogeneic hematopoietic cell transplantation in relapsed, refractory, and transformed indolent non-Hodgkin's lymphoma. J Clin Oncol. 2016. https://doi.org/10.1200/JCO.2007.11.5477.
53. Khouri IF, Saliba RM, Erwin WD, et al. Nonmyeloablative allogeneic transplantation with or without 90yttrium ibritumomab tiuxetan is potentially curative for relapsed follicular lymphoma: 12-year results. Blood. 2012;119(26):6373–8. https://doi.org/10.1182/blood-2012-03-417808.
54. Schouten HC, Qian W, Kvaloy S, et al. High-dose therapy improves progression-free survival and survival in relapsed follicular non-Hodgkin's lymphoma: results from the randomized European CUP trial. J Clin Oncol. 2003;21(21):3918–27. https://doi.org/10.1200/JCO.2003.10.023.
55. Rohatiner AZS, Nadler L, Davies AJ, et al. Myeloablative therapy with autologous bone marrow transplantation for follicular lymphoma at the time of second or subsequent remission: long-term follow-up. J Clin Oncol. 2007;25(18):2554–9. https://doi.org/10.1200/JCO.2006.09.8327.
56. Sebban C, Mounier N, Brousse N, et al. Standard chemotherapy with interferon compared with CHOP followed by high-dose therapy with autologous stem cell transplantation in untreated patients with advanced follicular lymphoma: the GELF-94 randomized study from the Groupe d'Etude des Lymphomes de l'Adulte (GELA). Blood. 2006;108(8):2540–4. https://doi.org/10.1182/blood-2006-03-013193.

57. Ladetto M, De Marco F, Benedetti F, et al. Prospective, multicenter randomized GITMO/IIL trial comparing intensive (R-HDS) versus conventional (CHOP-R) chemoimmunotherapy in high-risk follicular lymphoma at diagnosis: the superior disease control of R-HDS does not translate into an overall survival advantage. Blood. 2008;111(8):4004–13. https://doi.org/10.1182/blood-2007-10-116749.
58. Al Khabori M, de Almeida JR, Guyatt GH, Kuruvilla J, Crump M. Autologous stem cell transplantation in follicular lymphoma: a systematic review and meta-analysis. JNCI J Natl Cancer Inst. 2012;104(1):18–28. https://doi.org/10.1093/jnci/djr450.

Chapter 6
Mantle Cell Lymphoma: Therapeutics and Management

Edward A. Nabrinsky and Alexey V. Danilov

Introduction

Mantle cell lymphoma (MCL) is a rare subtype of non-Hodgkin lymphoma (NHL), which accounts for approximately 4% of lymphomas diagnosed in the United States; it is slightly more common in Western Europe [1, 2]. Most patients are diagnosed at the age of 60 or older, with median age at diagnosis of 68 years. Almost all cases of MCL harbor the chromosomal translocation t(11:14)(q13;32), which results in overexpression of cyclin D1, a diagnostic feature of this disease [3].

MCL typically follows an aggressive clinical course with an overall poor prognosis. Intensive chemo-immunotherapy (CIT) regimens remain the mainstay of treatment of newly diagnosed MCL. However, since many patients are older when diagnosed, they are poor candidates for such therapy due to the presence of medical comorbidities. Despite high initial response rates, most patients eventually relapse and succumb to their disease [4].

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Significant advances in diagnosis, risk stratification, and treatment of MCL were achieved in the past decade, leading to improved outcomes. First, several large clinical trials with extensive follow-up informed the choice of induction therapy in MCL. Second, rituximab maintenance has been cemented in the management of MCL. Third, introduction of agents interfering with B-cell receptor (BCR) signaling has revolutionized treatment of relapsed and refractory MCL. Finally, watch-and-wait strategy is now recognized as a valid approach in a subset of patients with MCL. This chapter will briefly summarize the standard of care options in management of MCL.

Watchful Waiting

Most patients with MCL require immediate therapy due to presence of debilitating symptoms, progressive lymphadenopathy, or cytopenias. However, a fraction of patients are diagnosed based on a routine complete blood count (lymphocytosis), during colonoscopy, or physical exam (lymphadenopathy) and are completely asymptomatic at initial presentation. Watchful waiting has been evaluated in MCL and has become an accepted strategy in such patients. A single-center retrospective analysis evaluated 97 newly diagnosed patients with MCL [5] and found no detriment to this approach, with a median overall survival (OS) not reached among those who deferred therapy. Calzada and colleagues conducted a multicenter retrospective analysis which included 395 patients with MCL, of which 72 (18%) received deferred therapy (defined as receipt of first treatment >90 days following initial diagnosis) [6]. In this study, patients receiving deferred therapy were more likely to have a good performance status with no B symptoms and a normal LDH level at diagnosis. Here too, deferred therapy was not associated with a significant difference in OS. Patients with leukemic non-nodal MCL (typically SOX11-negative, *IGHV*-mutated) [7] behave in a particularly indolent fashion, and studies show only one third (30%) require treatment within 3 years of diagnosis [8].

Frontline Management of MCL

CIT remains the standard approach to frontline management of MCL. For patients, the first decision is whether or not to pursue an intensive treatment approach. In general terms, older MCL patients should be spared intensive treatment approaches given the associated risks, whereas younger, fit patients are good candidates for intensive induction strategies [9]. While there is no firm rule defining the “older” and “younger” patients, many therapeutic trials have set an age cut-off of 65 years and are generally applied in our practice.

Radiotherapy

Given that the vast majority of patients present with either disseminated lymphadenopathy or with extensive extra-nodal disease, including involvement of the bone marrow, radiotherapy has very limited role in the management of previously untreated MCL, outside of palliation. However, MCL is very sensitive to this modality. Thus, in a rare patient who presents with truly localized disease, it is worth a consideration. International Lymphoma Radiation Oncology Group conducted a retrospective analysis of 179 patients with early-stage (stage I or II) MCL from 13 institutions [10]. The head and neck were the most common presenting sites (75%). The treatment outcomes were independent of treatment modality, with 10-year freedom from progression of approximately 40%. Most treatment failures occurred outside the original disease site. This study suggests that local radiation may be employed in patients with localized MCL to minimize the toxicity. However, it should be understood that such treatment approach is not curative.

The MCL International Prognostic Index (MIPI)

MIPI was developed in 2008 to help predict outcomes with CIT. Four hundred and fifty-five patients with de novo advanced-stage MCL were classified into low-, intermediate-, and high-risk groups based on four independent factors: leukocyte count, age, performance status, and lactate dehydrogenase (LDH) [11]. Subsequently, Ki-67 index was added to MIPI for better prognostication, where Ki-67 index >30% was associated with shorter OS and progression-free survival (PFS) [12].

Treatment of Younger Patients

Before the development of current treatment regimens, MCL was characterized by poor prognosis with low to moderate sensitivity to chemotherapy and a median survival of less than 5 years. Earlier drug combinations that included anthracyclines and purine analogues failed to improve long-term outcomes, despite demonstrating improved overall response rate (ORR) and complete remissions (CR) in a minority of cases. Addition of anti-CD20 antibody rituximab to CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) in patients with advanced-stage MCL resulted in improved CR, ORR, and time to treatment failure compared with CHOP alone [13]. A subsequent meta-analysis showed that rituximab combinations improved OS in patients with advanced-stage indolent lymphomas and MCL compared with chemotherapy [14].

Autologous hematopoietic stem cell transplant (auto-HSCT) has become a standard of care option for younger patients with MCL. The Nordic Lymphoma Group

(NLG) treated patients with advanced-stage MCL with four cycles of dose-intensified CHOP followed by a cytarabine-containing conditioning regimen and auto-HSCT. Eighty-five percent of patients failed therapy, and most had evidence of minimal residual disease after transplantation [15]. However, rituximab was not part of the induction regimen in this early trial. The subsequent NLG MCL-2 study incorporated high-dose cytarabine and rituximab into the induction regimen, along with dose-intensified CHOP. Among the 160 patients (65 years and younger), ORR and CR rates were 96% and 54%, respectively, and the 6-year OS and PFS rates were 70% and 66%, respectively [15]. A long-term follow-up report of NLG MCL-2 demonstrated a median OS and PFS of 12.7 and 8.5 years after a median follow-up of 11.4 years [16]. However, disease cures were not achieved with this regimen.

In the meantime, the Cancer and Leukemia Group B (CALGB) 55,909 study explored the effectiveness of auto-HSCT in patients up to the age of 69, with 2-year PFS as the primary endpoint. Treatment with an aggressive rituximab-containing CIT regimen using the methotrexate and CHOP backbone, followed by high doses of cytarabine combined with rituximab, demonstrated a 2-year PFS of 76% and a 2-year OS of 87% [17]. The above studies reflected a groundbreaking change in MCL treatment for young patients seeking aggressive regimens.

Following the CALBG study, a strategy was taken to explore high-dose cytarabine as part of an induction regimen in younger patients. This culminated in MCL Younger phase III trial, where 497 patients were randomized to receive either 6 courses of standard R-CHOP followed by an auto-HSCT, versus 6 courses of alternating R-CHOP or R-DHAP (rituximab plus high-dose dexamethasone, high-dose cytarabine, and cisplatin) followed by a high-dose cytarabine-containing conditioning regimen and auto-HSCT. The study demonstrated a median PFS of 9.1 years in the cytarabine group versus 3.9 years in the control group [18]. While the high-dose cytarabine group had an increased frequency of toxicities (hematologic, febrile neutropenia and renal) during the induction phase, the number of HSCT-related deaths was similar (3.4%). Hence, this trial endorsed high-dose cytarabine as part of the aggressive treatment among the stem cell transplant candidates.

Subsequently, a large cohort review of patients with chemotherapy-sensitive MCL who were HSCT recipients between 1996 and 2007 demonstrated that optimal timing for auto-HSCT is early in the disease course, particularly favorable if performed in first complete remission (CR1). For patients who received auto-HSCT after one prior line of chemotherapy, outcomes demonstrated 75% OS and 70% PFS at 5 years [19].

Given the efficacy of bendamustine in older patients with MCL, a phase II study employed rituximab/bendamustine (BR) for three cycles followed by rituximab/high-dose cytarabine for three additional cycles [20]. In this small study of 23 patients, 96% achieved CR, and 21 underwent auto-HSCT. This study positioned BR/cytarabine as a potential alternative to R-CHOP-based induction regimens in younger patients.

However, not all induction regimens may require consolidation with auto-HSCT. A phase II trial by Romaguera et al. utilized rituximab plus fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with

high-dose methotrexate-cytarabine (R-Hyper-CVAD/cytarabine-MTX) for 6–8 cycles in 97 patients with MCL. CR was 87%, and 3-year event-free survival was 73% among patients younger than 65 [21]. However, the regimen demonstrated significant toxicity, with resulting five deaths. Fifteen percent of treatment cycles were associated with neutropenic fever, three patients developed treatment-related myelodysplastic syndrome, and one patient developed acute myeloid leukemia. As a result, this regimen was not recommended for patients over 65 years old. A follow-up phase II multicenter study (SWOG 0213) sought to confirm the clinical efficacy of this regimen. The study included 49 patients with a median age of 57 years. Seven of the 49 patients were over age 65. When the analysis was limited to younger patients (≤65 years old), the median PFS was 5.5 years with a 5-year PFS which was 53% [22]. Of note, a similar toxicity profile was seen in comparison to the original study, with 39% of patients unable to complete all eight cycles due to toxicity.

The choice between the intensive induction regimens largely depends on patient's comorbidities, on the physician's experience, and the medical center preference.

Maintenance Rituximab Therapy in Younger Adults Post-HSCT

Lymphoma Study Association group (LYSA) trial conducted an investigation into the role of rituximab maintenance following auto-HSCT. This was a phase III study that randomized patients 65 years old or younger to receive rituximab maintenance therapy every 2 months for 3 years post-HSCT or observation [23]. With a median follow-up posttransplantation of 50.2 months, the PFS rate at 4 years was 83% in the rituximab group versus 64% in the observation group. OS was 89% versus 80%, respectively. This data highlighted that rituximab maintenance therapy post-HSCT increased OS and PFS in this population of patients. Furthermore, this trial has demonstrated that induction with four cycles of R-DHAP alone (without the R-CHOP component) is adequate induction prior to auto-HSCT.

Treatment of Older Adults with MCL

Older adults with good performance status are offered conventional CIT, of which there are several options. Patients who will end up with at least a PR following conventional CIT may be considered for auto-HSCT, if they are deemed candidates at that point. Those who are not candidates typically receive maintenance rituximab.

Bendamustine in combination with rituximab (BR) has shown fewer toxicities and somewhat superior efficacy in comparison with R-CHOP in MCL. A large phase III randomized STiL trial compared six cycles of BR ($n = 274$) to R-CHOP ($n = 275$), in patients with MCL and indolent non-Hodgkin lymphomas (18% of patients had MCL). BR resulted in prolonged median PFS in patients with MCL

(35.4 versus 22.1 months) and had lower rates of alopecia, hematologic toxicities, infection, and neuropathy [24]. The phase III BRIGHT study compared six cycles of BR to either standard R-CHOP or R- CVP (cyclophosphamide, vincristine, prednisone). Seventy-four of the 447 patients had MCL, and the rest had indolent non-Hodgkin lymphoma [25]. Over a median follow-up of 65 months, ORR for BR and R-CHOP/R-CVP were 94% and 85% among all patients and patients with MCL, respectively. BR was associated with a higher incidence of vomiting and hypersensitivity reactions but with lower rates of alopecia and peripheral neuropathy. As a result of the two trials, BR is often used as a first-line therapy choice due to its favorable response rates and acceptable toxicity profile in patients with MCL.

Cytarabine in combination with BR (R-BAC) was based on an earlier report that demonstrated a synergistic effect of bendamustine and cytarabine in preclinical studies in MCL [26]. The trial accrued 40 patients 65 years or older with either de novo or relapsed/refractory (after one prior CIT regimen) [27]. Treatment completion rate was 85%, with an ORR of 100% (95% CR for previously untreated patients) and a 2-year PFS of 95% among the previously untreated patients [26].

Following bortezomib approval in therapy of relapsed/refractory MCL, this drug was investigated in the frontline setting [28]. Four hundred and eighty-seven patients who were not eligible for auto-HSCT received 6–8 cycles of R-CHOP versus VR-CAP (vincristine, rituximab, cyclophosphamide, doxorubicin, prednisone) on a randomized phase III study. Median PFS was 24.7 months in the VR-CAP group versus 14.4 months in the R-CHOP group (HR 0.63, $P < 0.001$). CR rate was 53% versus 42%, and 4-year OS was 64% versus 54%, all favoring VR-CAP. While this improvement came at a cost of increased hematologic toxicity, with higher rates of neutropenia, leukopenia, and thrombocytopenia in the VR-CAP group, the number of completed cycles, treatment discontinuation, or deaths was not affected by adverse events. The results of this study suggest that VR-CAP can be used as frontline therapy in the elderly patients with newly diagnosed MCL. However, R-CHOP still remains an acceptable alternative.

Finally, the European MCL Network investigated whether maintenance therapy with rituximab or interferon-α following eight cycles of R-CHOP prolonged remission rates in patients with MCL [29]. Addition of rituximab reduced the risk of progression or death by 45%, with 58% of patients in remission after 4 years of therapy in comparison to 29% with interferon. Furthermore, maintenance with rituximab improved OS (87% versus 63%, $p = 0.005$). With BR's increasing popularity as an induction regimen among the elderly, Rummel and colleagues compared rituximab maintenance therapy versus observation after patients were treated with up to six cycles of BR plus two additional cycles of rituximab [30]. Median age was 70 years and median time of observation was 2 months. With the primary endpoint of PFS, no significant difference was observed. However, we still consider rituximab maintenance following induction therapy as a standard approach among the elderly patients with MCL.

Management of Relapsed MCL

Patients with relapsed MCL ultimately succumb to their disease. A number of CIT regimens have been employed in treatment of relapsed/refractory MCL, including BR, gemcitabine+oxaliplatin+rituximab, VR-CAP, R-CHOP, etc., with an ORR ranging between 50% and 90% and a PFS of 8–16 months [31].

In recent years, a number of agents enriched therapy of relapsed and refractory MCL. Single-agent bortezomib, a proteasome inhibitor, demonstrated efficacy in this setting, with ORR of 32% (CR/CRu 8%) and duration of response of 9.2 months [32], and has been since incorporated in combination chemotherapy regimens.

Temsirolimus, an inhibitor of mTOR signaling, was evaluated in a multicenter, phase III study of 162 patients with relapsed/refractory MCL in comparison with investigator's choice single-agent therapy. Temsirolimus was administered either 175 mg weekly for 3 weeks followed by either 75 mg (175/75-mg) or 25 mg (175/25-mg) weekly. Median PFS was 4.8, 3.4, and 1.9 months for the temsirolimus 175/75-mg, 175/25-mg, and the investigator's choice groups, respectively. ORR was 22% in the 175/75-mg group (2% in the investigator's choice arm) [33].

Lenalidomide, a pleiotropic pathway modifier, was combined with rituximab in a phase I/II trial of 52 patients with R/R MCL. Among 44 patients enrolled onto phase II part of the study, ORR was 57% (CR 36%). The median response duration was 18.9 months [34].

Targeting BCR Signaling

Therapeutic targeting of BTK has revolutionized treatment of relapsed/refractory MCL. Ibrutinib is a potent, orally bioavailable irreversible inhibitor of BTK that covalently binds the kinase active site. Ibrutinib received FDA approval in therapy of relapsed/refractory MCL based on the results of a phase II trial. One hundred and eleven patients with MCL who received a median of three prior therapies were treated with ibrutinib at 560 mg by mouth daily. Sixty-eight percent of patients achieved an ORR, and 21% entered CR (by Cheson criteria) [35]. Responses were seen irrespective of prior receipt of bortezomib. Duration of response was 13 months, and OS on study was less than 2 years [36].

Acalabrutinib is a second generation, selective BTK inhibitor which received an accelerated FDA approval for therapy of patients with relapsed/refractory MCL who received at least one prior therapy. One hundred and twenty-four patients with relapsed/refractory MCL and a median of two prior therapies were treated with acalabrutinib 100 mg by mouth twice daily [37]. ORR was 81%, with 43% of patients achieving a CR (by Lugano criteria). In this study, the median duration of response was 26 months, and the median PFS was 20 months. While PFS with acalabrutinib compares favorably with ibrutinib, patients who received acalabrutinib

were less heavily pre-treated (two vs three prior therapies). Furthermore, difference in response assessment could have accounted for higher CR rates.

Both ibrutinib and acalabrutinib have a distinct adverse event profile. Those include general adverse effects even such as diarrhea, arthralgias, rash, headaches, fatigue, and others [38]. Up to 12% of patients develop atrial fibrillation with ibrutinib, which is more frequent in older patients and in those who have history of atrial fibrillation [39, 40]. Bleeding is a concern, and patients who receive anticoagulation need to be carefully monitored [41]. While most adverse events happen in the first 6 months of treatment, hypertension can also develop late in patients who are treated with ibrutinib. Due to its relative selectivity, acalabrutinib may be associated with fewer adverse events, and patients who are intolerant of ibrutinib may still be good candidates for acalabrutinib therapy [42]. In a pooled analysis of safety data from seven clinical trials, acalabrutinib showed a tolerable safety profile in hematologic malignancies with most common adverse events being headache, diarrhea, fatigue, nausea, and bruising [43]. The most frequent grade ≥3 adverse events experienced were neutropenia (6.6%), anemia (1.5%), pneumonia (1.1%), and thrombocytopenia (1.5%).

Interestingly, outcomes with ibrutinib have been inferior among patients with comorbidities [44]. However, those agents remain a well-tolerated and effective therapeutic option in patients with MCL. Several other BTK inhibitors are currently undergoing clinical development in MCL. Examples include zanubrutinib (BGB-3111) and tirabrutinib (GS-4059) [45, 46].

Patients with MCL who develop resistance to ibrutinib have dismal outcomes [47, 48]. A large retrospective cohort study of 114 patients who progressed on ibrutinib documented an overall survival of <3 months following cessation of the drug [48]. Only a fraction of such patients responded to salvage therapy and nevertheless succumbed to disease within 6–8 months [47]. Similar outcomes have been reported in patients with chronic lymphocytic leukemia (CLL) and diffuse large B-cell lymphoma [49–51].

Mutations in BTK (C481S) and its downstream target phospholipase Cγ2 (PLCγ2) have been implicated in resistance to ibrutinib in CLL where they may account for the majority of resistant cases [52, 53]. In fact, emergence of BTK/PLCγ mutations typically predates clinical resistance in CLL [53]. By contrast, while such mutations are found in late ibrutinib failures in MCL, most patients relapse early in the course of therapy (within the first 6 months) and lack such mutations [48, 54]. Therefore, alternative mechanisms of resistance account for primary ibrutinib failure in MCL and may include upregulation of PI3K and NFκB pathways. Resistance to BTK inhibitors in MCL remains an unmet medical need.

PI3K (phosphoinotiside-3 kinase) inhibitors are a class of drugs which demonstrate activity in non-Hodgkin lymphoma. However, they show inferior efficacy in MCL compared with BTK inhibitors. In a phase I study of idelalisib, a PI3K-δ inhibitor, in patients with relapsed/refractory MCL, ORR was 40%, with a median PFS of 3.7 months [55]. Upregulation of PI-3Kα isoform may at least in part account for resistance to idelalisib in MCL [56]. Duvelisib, a PI3K-γδ inhibitor [57], and

copanlisib, a PI3K-αδ inhibitor [58], have received FDA approval for therapy of follicular lymphoma. However, there is limited information regarding activity of these agents in MCL. Combination therapy approaches will be necessary to enhance the efficacy of PI3K inhibitors in MCL.

Venetoclax, a BCL2 Inhibitor/BH3-mimetic

Venetoclax is a small molecule inhibitor of BCL2. Venetoclax received FDA approval in treatment of both de novo and relapsed/refractory CLL based on large randomized studies (MURANO, CLL14) [59, 60]. Twenty-eight patients with MCL were treated on a first-in-human phase I study of venetoclax 400 mg daily (after a ramp-up). Seventy-five percent of patients achieved a response, including 21% CR [61]. In patients with MCL, median PFS was 14 months; however patients did not receive prior BTK inhibitors [61]. Treatment-emergent adverse events were reported in 103 patients (97%), a majority of which were grade 1 to 2. Fifty-six percent of patients experienced grade 3–4 adverse events, predominantly hematologic, including anemia (15%), neutropenia (11%), and thrombocytopenia (9%). Given the risk of tumor lysis syndrome, patients with CLL and MCL who are treated with venetoclax required a dose ramp-up.

Venetoclax appears less effective in patients who progressed on ibrutinib therapy. In a retrospective analysis of 20 such patients, ORR was 53% (CR 18%), with the median PFS of 3.2 months [62].

A combination of ibrutinib and venetoclax has been explored in patients with MCL. Twenty-four patients were treated; 23 had relapsed/refractory MCL. In this study, the rate of CR was 71%; however it remains to be seen whether this therapeutic strategy will lead to durable responses [63].

Resistance to venetoclax is dependent on multiple mechanisms, including BCL2 mutations [64], mutations in components of SWI-SNF chromatin-remodeling complex [65], upregulation of alternative anti-apoptotic proteins (MCL1) [66–68], and others.

CNS Involvement in MCL

CNS involvement is rare in patients with de novo MCL. However, it is present in 4–6% of patients with relapsed MCL and is typically asymptomatic [69, 70]. Blastoid histology, presence of B symptoms, elevated lactate dehydrogenase, ECOG performance status ≥2, and a high MIPI score have been associated with CNS involvement. Outcomes are poor, with survival reported at 3.7 months [70]. Ibrutinib has been shown to cross blood-brain barrier and demonstrated activity in patients with MCL who have CNS involvement [71].

MCL with High-Risk Genetic Features

MCL with TP53 aberrations and complex karyotype represents an unmet medical need. In the European MCL-2 and MCL-3 trials, patients who carried TP53 mutations did not benefit from high-intensity chemotherapeutic regimens [72]. Meanwhile, a multicenter retrospective analysis identified complex karyotype as a predictor of poor outcomes in patients with MCL [73]. In this study, complex karyotype was associated with inferior OS (4.5 vs 11.6 years; $p < 0.01$) and PFS (1.9 vs 4.4 years; $p < 0.01$). Intensive induction and autologous stem cell transplantation did not neutralize this adverse effect. In our practice, we analyze all patients with MCL for presence of del(17p) (by FISH), TP53 mutations (by NGS), and complex karyotype. We rapidly transition such patients to novel therapies (BTK inhibitor).

Blastoid MCL remains poorly responsive to both standard CIT and novel targeted agents and represents an area of unmet clinical need.

References

1. Vose JM. Mantle cell lymphoma: 2017 update on diagnosis, risk-stratification, and clinical management. Am J Hematol. 2017;92(8):806–13. https://doi.org/10.1002/ajh.24797.
2. Romaguera JE, Fayad LE, Feng L, Hartig K, Weaver P, Rodriguez MA, et al. Ten-year follow-up after intense chemoimmunotherapy with Rituximab-HyperCVAD alternating with Rituximab-high dose methotrexate/cytarabine (R-MA) and without stem cell transplantation in patients with untreated aggressive mantle cell lymphoma. Br J Haematol. 2010;150(2):200–8. https://doi.org/10.1111/j.1365-2141.2010.08228.x.
3. Pal Singh S, Dammeijer F, Hendriks RW. Role of Bruton's tyrosine kinase in B cells and malignancies. Mol Cancer. 2018;17(1):57. https://doi.org/10.1186/s12943-018-0779-z.
4. Stephens DM, Spurgeon SE. Ibrutinib in mantle cell lymphoma patients: glass half full? Evidence and opinion. Ther Adv Hematol. 2015;6(5):242–52. https://doi.org/10.1177/2040620715592569.
5. Martin P, Chadburn A, Christos P, Weil K, Furman RR, Ruan J, et al. Outcome of deferred initial therapy in mantle-cell lymphoma. J Clin Oncol. 2009;27(8):1209–13. https://doi.org/10.1200/JCO.2008.19.6121.
6. Calzada O, Switchenko JM, Maly JJ, Blum KA, Grover N, Mathews S, et al. Deferred treatment is a safe and viable option for selected patients with mantle cell lymphoma. Leuk Lymphoma. 2018;59(12):2862–70. https://doi.org/10.1080/10428194.2018.1455973.
7. Puente XS, Jares P, Campo E. Chronic lymphocytic leukemia and mantle cell lymphoma: crossroads of genetic and microenvironment interactions. Blood. 2018;131(21):2283–96. https://doi.org/10.1182/blood-2017-10-764373.
8. Clot G, Jares P, Gine E, Navarro A, Royo C, Pinyol M, et al. A gene signature that distinguishes conventional and leukemic nonnodal mantle cell lymphoma helps predict outcome. Blood. 2018;132(4):413–22. https://doi.org/10.1182/blood-2018-03-838136.
9. Fakhri B, Kahl B. Current and emerging treatment options for mantle cell lymphoma. Ther Adv Hematol. 2017;8(8):223–34. https://doi.org/10.1177/2040620717719616.
10. Dabaja BS, Zelenetz AD, Ng AK, Tsang RW, Qi S, Allen PK, et al. Early-stage mantle cell lymphoma: a retrospective analysis from the International Lymphoma Radiation Oncology Group (ILROG). Ann Oncol. 2017;28(9):2185–90. https://doi.org/10.1093/annonc/mdx334.

11. Hoster E, Dreyling M, Klapper W, Gisselbrecht C, van Hoof A, Kluin-Nelemans HC, et al. A new prognostic index (MIPI) for patients with advanced-stage mantle cell lymphoma. Blood. 2008;111(2):558–65. https://doi.org/10.1182/blood-2007-06-095331.
12. Hoster E, Rosenwald A, Berger F, Bernd HW, Hartmann S, Loddenkemper C, et al. Prognostic value of Ki-67 index, cytology, and growth pattern in mantle-cell lymphoma: results from randomized trials of the European mantle cell lymphoma network. J Clin Oncol. 2016;34(12):1386–94. https://doi.org/10.1200/jco.2015.63.8387.
13. Lenz G, Dreyling M, Hoster E, Wormann B, Duhrsen U, Metzner B, et al. Immunochemotherapy with rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone significantly improves response and time to treatment failure, but not long-term outcome in patients with previously untreated mantle cell lymphoma: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group (GLSG). J Clin Oncol. 2005;23(9):1984–92. https://doi.org/10.1200/JCO.2005.08.133.
14. Schulz H, Bohlius JF, Trelle S, Skoetz N, Reiser M, Kober T, et al. Immunochemotherapy with rituximab and overall survival in patients with indolent or mantle cell lymphoma: a systematic review and meta-analysis. J Natl Cancer Inst. 2007;99(9):706–14. https://doi.org/10.1093/jnci/djk152.
15. Geisler CH, Kolstad A, Laurell A, Andersen NS, Pedersen LB, Jerkeman M, et al. Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo–purged stem cell rescue: a nonrandomized phase 2 multicenter study by the Nordic Lymphoma Group. Blood. 2008;112(7):2687. https://doi.org/10.1182/blood-2008-03-147025.
16. Eskelund CW, Kolstad A, Jerkeman M, Raty R, Laurell A, Eloranta S, et al. 15-year follow-up of the Second Nordic Mantle Cell Lymphoma trial (MCL2): prolonged remissions without survival plateau. Br J Haematol. 2016;175(3):410–8. https://doi.org/10.1111/bjh.14241.
17. Damon LE, Johnson JL, Niedzwiecki D, Cheson BD, Hurd DD, Bartlett NL, et al. Immunochemotherapy and autologous stem-cell transplantation for untreated patients with mantle-cell lymphoma: CALGB 59909. J Clin Oncol. 2009;27(36):6101–8. https://doi.org/10.1200/JCO.2009.22.2554.
18. Hermine O, Hoster E, Walewski J, Bosly A, Stilgenbauer S, Thieblemont C, et al. Addition of high-dose cytarabine to immunochemotherapy before autologous stem-cell transplantation in patients aged 65 years or younger with mantle cell lymphoma (MCL Younger): a randomised, open-label, phase 3 trial of the European Mantle Cell Lymphoma Network. Lancet. 2016;388(10044):565–75. https://doi.org/10.1016/s0140-6736(16)00739-x.
19. Fenske TS, Zhang MJ, Carreras J, Ayala E, Burns LJ, Cashen A, et al. Autologous or reduced-intensity conditioning allogeneic hematopoietic cell transplantation for chemotherapy-sensitive mantle-cell lymphoma: analysis of transplantation timing and modality. J Clin Oncol. 2014;32(4):273–81. https://doi.org/10.1200/jco.2013.49.2454.
20. Armand P, Redd R, Bsat J, Mayuram S, Giardino A, Fisher DC, et al. A phase 2 study of Rituximab-Bendamustine and Rituximab-Cytarabine for transplant-eligible patients with mantle cell lymphoma. Br J Haematol. 2016;173(1):89–95. https://doi.org/10.1111/bjh.13929.
21. Romaguera JE, Fayad L, Rodriguez MA, Broglio KR, Hagemeister FB, Pro B, et al. High rate of durable remissions after treatment of newly diagnosed aggressive mantle-cell lymphoma with rituximab plus hyper-CVAD alternating with rituximab plus high-dose methotrexate and cytarabine. J Clin Oncol. 2005;23(28):7013–23. https://doi.org/10.1200/jco.2005.01.1825.
22. Bernstein SH, Epner E, Unger JM, Leblanc M, Cebula E, Burack R, et al. A phase II multicenter trial of hyperCVAD MTX/Ara-C and rituximab in patients with previously untreated mantle cell lymphoma; SWOG 0213. Ann Oncol. 2013;24(6):1587–93. https://doi.org/10.1093/annonc/mdt070.
23. Le Gouill S, Thieblemont C, Oberic L, Moreau A, Bouabdallah K, Dartigeas C, et al. Rituximab after autologous stem-cell transplantation in mantle-cell lymphoma. N Engl J Med. 2017;377(13):1250–60. https://doi.org/10.1056/NEJMoa1701769.

24. Rummel MJ, Niederle N, Maschmeyer G, Banat GA, von Grunhagen U, Losem C, et al. Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. Lancet. 2013;381(9873):1203–10. https://doi.org/10.1016/S0140-6736(12)61763-2.
25. Flinn IW, van der Jagt R, Kahl BS, Wood P, Hawkins TE, Macdonald D, et al. Randomized trial of bendamustine-rituximab or R-CHOP/R-CVP in first-line treatment of indolent NHL or MCL: the BRIGHT study. Blood. 2014;123(19):2944–52. https://doi.org/10.1182/blood-2013-11-531327.
26. Visco C, Castegnaro S, Chieregato K, Bernardi M, Albiero E, Zanon C, et al. The cytotoxic effects of bendamustine in combination with cytarabine in mantle cell lymphoma cell lines. Blood Cells Mol Dis. 2012;48(1):68–75. https://doi.org/10.1016/j.bcmd.2011.09.007.
27. Visco C, Finotto S, Zambello R, Paolini R, Menin A, Zanotti R, et al. Combination of rituximab, bendamustine, and cytarabine for patients with mantle-cell non-Hodgkin lymphoma ineligible for intensive regimens or autologous transplantation. J Clin Oncol. 2013;31(11):1442–9. https://doi.org/10.1200/JCO.2012.45.9842.
28. Robak T, Huang H, Jin J, Zhu J, Liu T, Samoilova O, et al. Bortezomib-based therapy for newly diagnosed mantle-cell lymphoma. N Engl J Med. 2015;372(10):944–53. https://doi.org/10.1056/NEJMoa1412096.
29. Kluin-Nelemans HC, Hoster E, Hermine O, Walewski J, Trneny M, Geisler CH, et al. Treatment of older patients with mantle-cell lymphoma. N Engl J Med. 2012;367(6):520–31. https://doi.org/10.1056/NEJMoa1200920.
30. Rummel MJ, Knauf W, Goerner M, Soeling U, Lange E, Hertenstein B, et al. Two years rituximab maintenance vs. observation after first-line treatment with bendamustine plus rituximab (B-R) in patients with mantle cell lymphoma: first results of a prospective, randomized, multicenter phase II study (a subgroup study of the StiL NHL7-2008 MAINTAIN trial). J Clin Oncol. 2016;34(15_suppl):7503. https://doi.org/10.1200/JCO.2016.34.15_suppl.7503.
31. Maddocks K. Update on mantle cell lymphoma. Blood. 2018;132(16):1647–56. https://doi.org/10.1182/blood-2018-03-791392.
32. Goy A, Bernstein SH, Kahl BS, Djulbegovic B, Robertson MJ, de Vos S, et al. Bortezomib in patients with relapsed or refractory mantle cell lymphoma: updated time-to-event analyses of the multicenter phase 2 PINNACLE study. Ann Oncol. 2009;20(3):520–5. https://doi.org/10.1093/annonc/mdn656.
33. Hess G, Herbrecht R, Romaguera J, Verhoef G, Crump M, Gisselbrecht C, et al. Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. J Clin Oncol. 2009;27(23):3822–9. https://doi.org/10.1200/JCO.2008.20.7977.
34. Wang M, Fayad L, Wagner-Bartak N, Zhang L, Hagemeister F, Neelapu SS, et al. Lenalidomide in combination with rituximab for patients with relapsed or refractory mantle-cell lymphoma: a phase 1/2 clinical trial. Lancet Oncol. 2012;13(7):716–23. https://doi.org/10.1016/S1470-2045(12)70200-0.
35. Wang ML, Rule S, Martin P, Goy A, Auer R, Kahl BS, et al. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. N Engl J Med. 2013;369(6):507–16. https://doi.org/10.1056/NEJMoa1306220.
36. Wang ML, Blum KA, Martin P, Goy A, Auer R, Kahl BS, et al. Long-term follow-up of MCL patients treated with single-agent ibrutinib: updated safety and efficacy results. Blood. 2015;126(6):739–45. https://doi.org/10.1182/blood-2015-03-635326.
37. Wang M, Rule S, Zinzani PL, Goy A, Casasnovas O, Smith SD, et al. Long-term follow-up of acalabrutinib monotherapy in patients with relapsed/refractory mantle cell lymphoma. Blood. 2018;132(Suppl 1):2876. https://doi.org/10.1182/blood-2018-99-110327.
38. Brown JR. How I treat CLL patients with ibrutinib. Blood. 2018;131(4):379–86. https://doi.org/10.1182/blood-2017-08-764712.

39. Brown JR, Moslehi J, O'Brien S, Ghia P, Hillmen P, Cymbalista F, et al. Characterization of atrial fibrillation adverse events reported in ibrutinib randomized controlled registration trials. Haematologica. 2017;102(10):1796–805. https://doi.org/10.3324/haematol.2017.171041.
40. Wiczer TE, Levine LB, Brumbaugh J, Coggins J, Zhao Q, Ruppert AS, et al. Cumulative incidence, risk factors, and management of atrial fibrillation in patients receiving ibrutinib. Blood Adv. 2017;1(20):1739–48. https://doi.org/10.1182/bloodadvances.2017009720.
41. Shatzel JJ, Olson SR, Tao DL, McCarty OJ, Danilov AV, DeLoughery TG. Ibrutinib-associated bleeding: pathogenesis, management, and risk reduction strategies. J Thromb Haemost. 2017. https://doi.org/10.1111/jth.13651.
42. Awan FT, Schuh A, Brown JR, Furman RR, Pagel JM, Hillmen P, et al. Acalabrutinib monotherapy in patients with chronic lymphocytic leukemia who are intolerant to ibrutinib. Blood Adv. 2019;3(9):1553–62. https://doi.org/10.1182/bloodadvances.2018030007.
43. Byrd JC, Owen R, O'Brien SM, Brown JR, Hillmen P, Bitman B, et al. Pooled analysis of safety data from clinical trials evaluating acalabrutinib monotherapy in hematologic malignancies. Blood. 2017;130(Suppl 1):4326.
44. Gordon MJ, Churnetski M, Alqahtani H, Rivera X, Kittai A, Amrock SM, et al. Comorbidities predict inferior outcomes in chronic lymphocytic leukemia treated with ibrutinib. Cancer. 2018;124(15):3192–200. https://doi.org/10.1002/cncr.31554.
45. Tarantelli C, Zhang L, Curti E, Gaudio E, Spriano F, Priebe V, et al. The Bruton tyrosine kinase inhibitor zanubrutinib (BGB-3111) demonstrated synergies with other anti-lymphoma targeted agents. Haematologica. 2019;104(7):e307–e9. https://doi.org/10.3324/haematol.2018.214759.
46. Walter HS, Rule SA, Dyer MJ, Karlin L, Jones C, Cazin B, et al. A phase 1 clinical trial of the selective BTK inhibitor ONO/GS-4059 in relapsed and refractory mature B-cell malignancies. Blood. 2016;127(4):411–9. https://doi.org/10.1182/blood-2015-08-664086.
47. Cheah CY, Chihara D, Romaguera JE, Fowler NH, Seymour JF, Hagemeister FB, et al. Patients with mantle cell lymphoma failing ibrutinib are unlikely to respond to salvage chemotherapy and have poor outcomes. Ann Oncol. 2015;26(6):1175–9. https://doi.org/10.1093/annonc/mdv111.
48. Martin P, Maddocks K, Leonard JP, Ruan J, Goy A, Wagner-Johnston N, et al. Postibrutinib outcomes in patients with mantle cell lymphoma. Blood. 2016;127(12):1559–63. https://doi.org/10.1182/blood-2015-10-673145.
49. Jain P, Keating M, Wierda W, Estrov Z, Ferrajoli A, Jain N, et al. Outcomes of patients with chronic lymphocytic leukemia after discontinuing ibrutinib. Blood. 2015;125(13):2062–7. https://doi.org/10.1182/blood-2014-09-603670.
50. Maddocks KJ, Ruppert AS, Lozanski G, Heerema NA, Zhao W, Abruzzo L, et al. Etiology of ibrutinib therapy discontinuation and outcomes in patients with chronic lymphocytic leukemia. JAMA Oncol. 2015;1(1):80–7. https://doi.org/10.1001/jamaoncol.2014.218.
51. Wilson WH, Young RM, Schmitz R, Yang Y, Pittaluga S, Wright G, et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. Nat Med. 2015;21(8):922–6. https://doi.org/10.1038/nm.3884.
52. Woyach JA, Furman RR, Liu TM, Ozer HG, Zapatka M, Ruppert AS, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. N Engl J Med. 2014. https://doi.org/10.1056/NEJMoa1400029.
53. Woyach JA, Ruppert AS, Guinn D, Lehman A, Blachly JS, Lozanski A, et al. BTKC481S-mediated resistance to ibrutinib in chronic lymphocytic leukemia. J Clin Oncol. 2017;35(13):1437–43. https://doi.org/10.1200/JCO.2016.70.2282.
54. Chiron D, Di Liberto M, Martin P, Huang X, Sharman J, Blecua P, et al. Cell-cycle reprogramming for PI3K inhibition overrides a relapse-specific C481S BTK mutation revealed by longitudinal functional genomics in mantle cell lymphoma. Cancer Discov. 2014;4(9):1022–35. https://doi.org/10.1158/2159-8290.CD-14-0098.
55. Kahl BS, Spurgeon SE, Furman RR, Flinn IW, Coutre SE, Brown JR, et al. A phase 1 study of the PI3Kdelta inhibitor idelalisib in patients with relapsed/refractory mantle cell lymphoma (MCL). Blood. 2014;123(22):3398–405. https://doi.org/10.1182/blood-2013-11-537555.

56. Iyengar S, Clear A, Bodor C, Maharaj L, Lee A, Calaminici M, et al. P110alpha-mediated constitutive PI3K signaling limits the efficacy of p110delta-selective inhibition in mantle cell lymphoma, particularly with multiple relapse. Blood. 2013;121(12):2274–84. https://doi.org/10.1182/blood-2012-10-460832.
57. Flinn IW, Miller CB, Ardeshna KM, Tetreault S, Assouline SE, Mayer J, et al. DYNAMO: a phase II study of Duvelisib (IPI-145) in patients with refractory indolent non-Hodgkin lymphoma. J Clin Oncol. 2019;37(11):912–22. https://doi.org/10.1200/JCO.18.00915.
58. Dreyling M, Santoro A, Mollica L, Leppa S, Follows GA, Lenz G, et al. Phosphatidylinositol 3-kinase inhibition by copanlisib in relapsed or refractory indolent lymphoma. J Clin Oncol. 2017;35(35):3898–905. https://doi.org/10.1200/JCO.2017.75.4648.
59. Fischer K, Al-Sawaf O, Bahlo J, Fink AM, Tandon M, Dixon M, et al. Venetoclax and obinutuzumab in patients with CLL and coexisting conditions. N Engl J Med. 2019;380(23):2225–36. https://doi.org/10.1056/NEJMoa1815281.
60. Seymour JF, Kipps TJ, Eichhorst B, Hillmen P, D'Rozario J, Assouline S, et al. Venetoclax-rituximab in relapsed or refractory chronic lymphocytic leukemia. N Engl J Med. 2018;378(12):1107–20. https://doi.org/10.1056/NEJMoa1713976.
61. Davids MS, Roberts AW, Seymour JF, Pagel JM, Kahl BS, Wierda WG, et al. Phase I first-in-human study of venetoclax in patients with relapsed or refractory non-Hodgkin lymphoma. J Clin Oncol. 2017;35(8):826–33. https://doi.org/10.1200/JCO.2016.70.4320.
62. Eyre TA, Walter HS, Iyengar S, Follows G, Cross M, Fox CP, et al. Efficacy of venetoclax monotherapy in patients with relapsed, refractory mantle cell lymphoma post BTK inhibitor therapy. Haematologica. 2018. https://doi.org/10.3324/haematol.2018.198812.
63. Tam CS, Anderson MA, Pott C, Agarwal R, Handunnetti S, Hicks RJ, et al. Ibrutinib plus venetoclax for the treatment of mantle-cell lymphoma. N Engl J Med. 2018;378(13):1211–23. https://doi.org/10.1056/NEJMoa1715519.
64. Blombery P, Anderson MA, Gong JN, Thijssen R, Birkinshaw RW, Thompson ER, et al. Acquisition of the recurrent Gly101Val mutation in BCL2 confers resistance to venetoclax in patients with progressive chronic lymphocytic leukemia. Cancer Discov. 2019;9(3):342–53. https://doi.org/10.1158/2159-8290.CD-18-1119.
65. Agarwal R, Chan YC, Tam CS, Hunter T, Vassiliadis D, Teh CE, et al. Dynamic molecular monitoring reveals that SWI-SNF mutations mediate resistance to ibrutinib plus venetoclax in mantle cell lymphoma. Nat Med. 2019;25(1):119–29. https://doi.org/10.1038/s41591-018-0243-z.
66. Bojarczuk K, Sasi BK, Gobessi S, Innocenti I, Pozzato G, Laurenti L, et al. BCR signaling inhibitors differ in their ability to overcome Mcl-1-mediated resistance of CLL B cells to ABT-199. Blood. 2016;127(25):3192–201. https://doi.org/10.1182/blood-2015-10-675009.
67. Thijssen R, Slinger E, Weller K, Geest CR, Beaumont T, van Oers MH, et al. Resistance to ABT-199 induced by microenvironmental signals in chronic lymphocytic leukemia can be counteracted by CD20 antibodies or kinase inhibitors. Haematologica. 2015;100(8):e302–6. https://doi.org/10.3324/haematol.2015.124560.
68. Tromp JM, Geest CR, Breij EC, Elias JA, van Laar J, Luijks DM, et al. Tipping the Noxa/Mcl-1 balance overcomes ABT-737 resistance in chronic lymphocytic leukemia. Clin Cancer Res. 2012;18(2):487–98. https://doi.org/10.1158/1078-0432.CCR-11-1440.
69. Gill S, Herbert KE, Prince HM, Wolf MM, Wirth A, Ryan G, et al. Mantle cell lymphoma with central nervous system involvement: frequency and clinical features. Br J Haematol. 2009;147(1):83–8. https://doi.org/10.1111/j.1365-2141.2009.07835.x.
70. Cheah CY, George A, Gine E, Chiappella A, Kluin-Nelemans HC, Jurczak W, et al. Central nervous system involvement in mantle cell lymphoma: clinical features, prognostic factors and outcomes from the European Mantle Cell Lymphoma Network. Ann Oncol. 2013;24(8):2119–23. https://doi.org/10.1093/annonc/mdt139.
71. Bernard S, Goldwirt L, Amorim S, Brice P, Briere J, de Kerviler E, et al. Activity of ibrutinib in mantle cell lymphoma patients with central nervous system relapse. Blood. 2015;126(14):1695–8. https://doi.org/10.1182/blood-2015-05-647834.

72. Eskelund CW, Dahl C, Hansen JW, Westman M, Kolstad A, Pedersen LB, et al. TP53 mutations identify younger mantle cell lymphoma patients who do not benefit from intensive chemoimmunotherapy. Blood. 2017;130(17):1903–10. https://doi.org/10.1182/blood-2017-04-779736.
73. Greenwell IB, Staton AD, Lee MJ, Switchenko JM, Saxe DF, Maly JJ, et al. Complex karyotype in patients with mantle cell lymphoma predicts inferior survival and poor response to intensive induction therapy. Cancer. 2018;124(11):2306–15. https://doi.org/10.1002/cncr.31328.

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